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PATHOLOGY OF THE CENTRAL NERVOUS SYSTEM IN CANINE BLACK TONGUE

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The etiology of pellagra, which was apparently established by Goldberger on the basis of a vitamin deficiency, has become once more a matter of controversy. This is largely due to the unsatisfactory results of therapy, for not infrequently the oral use of materials rich in vitamin B complex and hence presumably containing the pellagra-preventing factor described by Goldberger and his co-workers fails to cure. The work of that group was based, to a large degree, on studies of an experimentally induced condition in dogs known as black tongue. This canine disease is marked by stomatitis, glossitis, salivation and diarrhea, all these symptoms being prominent also in pellagra. According to Wheeler, black tongue and pellagra are the same disease, "on account of their seasonal and geographical incidence, their common cause and similar course, their identical pathological changes and their equal response to the same therapeutic and preventive measures." Analysis of the published studies, however, shows that in one pathologic respect the two conditions cannot be stated to be identical. The discrepancy is in the presence of pathologic alterations of the central nervous system in pellagra and their absence in black tongue. This discrepancy is the more important because of recent studies showing the effect of lack of the vitamin B complex on the production of lesions of the central nervous system marked by loss of myelin. If, as is claimed by the Goldberger school, pellagra is due to the absence of the thermostabile fraction of the vitamin B complex and if such a lack is causative in the production of demyelinating lesions of the central nervous system, both black tongue and pellagra should be associated with pathologic alterations of the brain and spinal cord. A large number of histologic studies have given incontrovertible proof that degenerative lesions both of myelin and of nerve cells are present in pellagra. The prominence in pellagra of symptoms referable to nerve lesions is further evidence of involvement of the

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central nervous system. It is difficult to reconcile the lack of parallelism in the neuropathologic studies of black tongue and pellagra in view of the remarkable pathologic and symptomatic similarity in other respects.

Since the studies of the neuropathology of canine black tongue are not extensive or detailed, changes similar to those found in the central nervous systems of persons dying of pellagra may have been overlooked. Accordingly, the brains and spinal cords of twelve dogs dying of acute black tongue were studied by modern neuropathologic methods. The changes observed were similar in many respects to those seen in pellagra. These changes were also similar to those described as occurring in animals kept on diets deficient in the vitamin B complex. The presence of degenerative lesions of the central nervous system in pellagra may explain, to a certain extent, the difficulties attending therapy in pellagra. These pathologic alterations also make up a further link in the chain of evidence associating deficiency in a factor closely allied to the vitamin B complex with the etiologic agent of pellagra, black tongue and certain conditions in man marked by loss of myelin in the central nervous system.

REVIEW OF THE LITERATURE

The only comprehensive and detailed studies of the pathologic changes in acute black tongue are those of Denton.[1a] The animals were killed and examined immediately. The nervous systems were studied by general pathologic methods used as a routine. Aside from alterations of the nerve cells, no significant observations were reported.

The literature on pellagra contains a number of references to alterations of the central nervous system. Denton [1b] observed only indefinite changes in the nerve cells in the more acute cases. Singer and Pollock [2] described chromatolysis of nerve cells, astrocytosis and satellitosis, as well as destruction of nerve fibers. Langworthy [3] observed diffuse loss of nerve fibers and changes in the nerve cell. Castellani and Chalmers [4] observed degeneration of nerve cells and disappearance of fibrils in the posterior and lateral columns. Singer [5] observed diffuse degeneration of fibers in the white matter and pronounced changes in the nerve cells of the cerebrum and cord. Sclerosis was observed in the more chronic cases. Klauder and Winkelman [6] described so-called "central neuritis"

1. Denton, J.: (*a*) Am. J. Path. **4**:341, 1928; (*b*) Am. J. Trop. Med. **5**:173, 1925.

2. Singer, H. D., and Pollock, L. J.: Arch. Int. Med. **11**:565, 1913.

3. Langworthy, O. R.: Brain **54**:291, 1931.

4. Castellani, A., and Chalmers, A. J.: Manual of Tropical Medicine, ed. 3, London, Baillière, Tindall & Cox, 1919.

5. Singer, H. D.: Arch. Int. Med. **15**:121, 1915.

6. Klauder, J. V., and Winkelman, N. W.: J. A. M. A. **90**:364, 1928.

in the brains of twelve patients with pellagra. The pathologic changes included swelling of the cells, with disappearance of Nissl bodies, and an increased lipoid content throughout the entire central nervous system. Wilson [7] observed chromatolysis of the nerve cells of Clarke's column and the anterior horns, as well as degeneration of the white matter. Vedder [8] described extensive myelin degeneration of the cord, with chromatolysis and pigmentation of nerve cells. Kozowsky [9] and Winkelman [10] observed a deposit of fat in the nerve cells in pellagra, as did Sandwith [11] and Pentschew.[12] Reed and Ash [13] described column degeneration of the spinal cord in sprue, a condition having certain symptomatic similarities to pellagra. The etiologic relationship of deficiencies in the water-soluble vitamin to pathologic alterations in the central nervous system has been observed since the early studies of beriberi.[14] More recent investigation employing mammals has brought forward additional evidence. Gildea, Kattwinkel and Castle [15] made histologic studies of the central nervous systems of dogs maintained on a synthetic diet deficient in the vitamin B complex. Extensive degenerative changes in the myelin sheaths were observed. Faulty staining technic made their results difficult to interpret. It does not appear, however, that lesions followed deprivation of any single component of the vitamin B complex. Zimmerman and Burack [16] described lesions of myelin in animals maintained on diets deficient in the thermolabile, and probably also in the thermostabile, fractions of the vitamin B complex. The evidence is clear that lack of the water-soluble vitamin gives rise to demyelinating lesions of the central nervous system, but the evidence for the rôle of any particular component of the vitamin B complex is as yet unsatisfactory.

METHODS

Dogs of good size and of mongrel breed, largely short-haired, were employed. The animals were kept under uniform conditions in individual cages with bedding

7. Wilson, S. A. K.: Proc. Roy. Soc. Med. (Neurol. Sect.) **7**:31, 1914.
8. Vedder, E. B.: Arch. Int. Med. **18**:137, 1916.
9. Kozowsky, A. D.: Arch. f. Psychiat. **49**:204, 556 and 873, 1911-1912.
10. Winkelman, N. W.: Ztschr. f. d. ges. Neurol. u. Psychiat. **102**:38, 1926.
11. Sandwith, F. M.: J. Path. & Bact. **7**:460, 1901.
12. Pentschew, A.: Ztschr. f. d. ges. Neurol. u. Psychiat. **118**:17, 1928.
13. Reed, A. C., and Ash, J. E.: Arch. Int. Med. **40**:787, 1927.
14. (*a*) Eijkman, C.: Virchows Arch. f. path. Anat. **148**:523, 1897; (*b*) Vedder, E. B., and Clark, E.: Philippine J. Sc. **7**:423, 1912; (*c*) Findlay, G. M.: J. Path. & Bact. **24**:175, 1921.
15. Gildea, E. F.; Kattwinkel, E. E., and Castle, W. B.: New England J. Med. **202**:523, 1930.
16. Zimmerman, H. M., and Burack, E.: Arch. Path. **13**:207, 1932.

of shavings. No particular care was taken to avoid coprophagy. The diet was composed of the following ingredients:

White corn-meal	6,000 Gm.
California black-eyed peas	750 Gm.
Casein	900 Gm.
Cod liver oil	450 cc.
Cottonseed oil	450 cc.
Rice polishings	600 Gm.
Calcium carbonate	450 Gm.
Sodium chloride	150 Gm.

The corn-meal, peas and casein were mixed and cooked for two hours in a steam cooker. The remaining ingredients were then added and thoroughly mixed. The dogs were fed six days a week and were allowed to eat as much as they chose. They were weighed at weekly intervals.

Treatment was instituted only when the symptoms were so acute and severe that it seemed doubtful whether the animal would survive in case no therapy was given. In treatment, 200 Gm. of raw beef was fed daily if the animal would eat. If not, a rice polishings concentrate prepared according to the method described by Evans and Lepovsky [17] was administered by stomach tube. Occasionally a brewers' yeast concentrate was given. Successful therapeutic results were so difficult to obtain when the disease was at its height that it was often impossible to confine the treatment to some simple material.

In general, the diet was well taken. Control animals which were given 4 Gm. daily of liver extract-Lilly ate no more than the dogs maintained on the unsupplemented diet and remained in perfect health.

Animals which ate sparingly of the special diet also failed to acquire the disease under investigation, but frequently died of intercurrent infections. It seemed clear that whatever symptoms developed were due to the absence of an accessory food factor in the diet and not to lack of salts, metallic elements, particular proteins or caloric intake.

Staining Technic.—Autopsies were done on seven of the animals within one-half hour after death. In the other five the period post mortem was not known exactly, since the bodies were found in the morning. The brains and spinal cords were fixed at autopsy in a diluted solution of formaldehyde, U.S.P. (1:10). From three to five months after the death of the dogs the material was sent to the Neurological Laboratory of the Boston City Hospital. Blocks were cut at once and put into Müller's solution [17a] for staining by the Weigert method. After six weeks in Müller's solution the blocks were embedded in pyroxylin (celloidin) and cut and stained by the conventional Weigert-Pal technic. Other blocks were washed and passed through alcohol and pyroxylin. Sections from these blocks were stained with toluidine blue. Stains for fat were made on frozen sections by the Herxheimer method, using scarlet red. For the Alzheimer-Mann stains, blocks were cut and mordanted in "Gliabeize." [17b] Mann's solution (equal parts

17. Evans, H. M., and Lepovsky, J.: J. Nutrition **3**:353, 1931.

17a. Müller's solution is: potassium bichromate, 2 Gm.; sodium sulphate, 1 Gm., and water, 100 cc.

17b. The formula for Gliabeize is as follows: potassium bichromate, 5 Gm.; fluorochrome, 2 Gm., and water, 100 cc. (Mallory, F. B., and Wright, J. H.: Pathological Technique, ed. 8, Philadelphia, W. B. Saunders Company, 1924, p. 155).

of eosin and methyl blue) was used for staining the frozen sections. This method stains normal axis-cylinders a clear blue, in striking contrast to the red myelin and stroma.

Examination of the sections stained by the Weigert method disclosed widespread changes; hence, other stains were employed in an attempt to confirm the

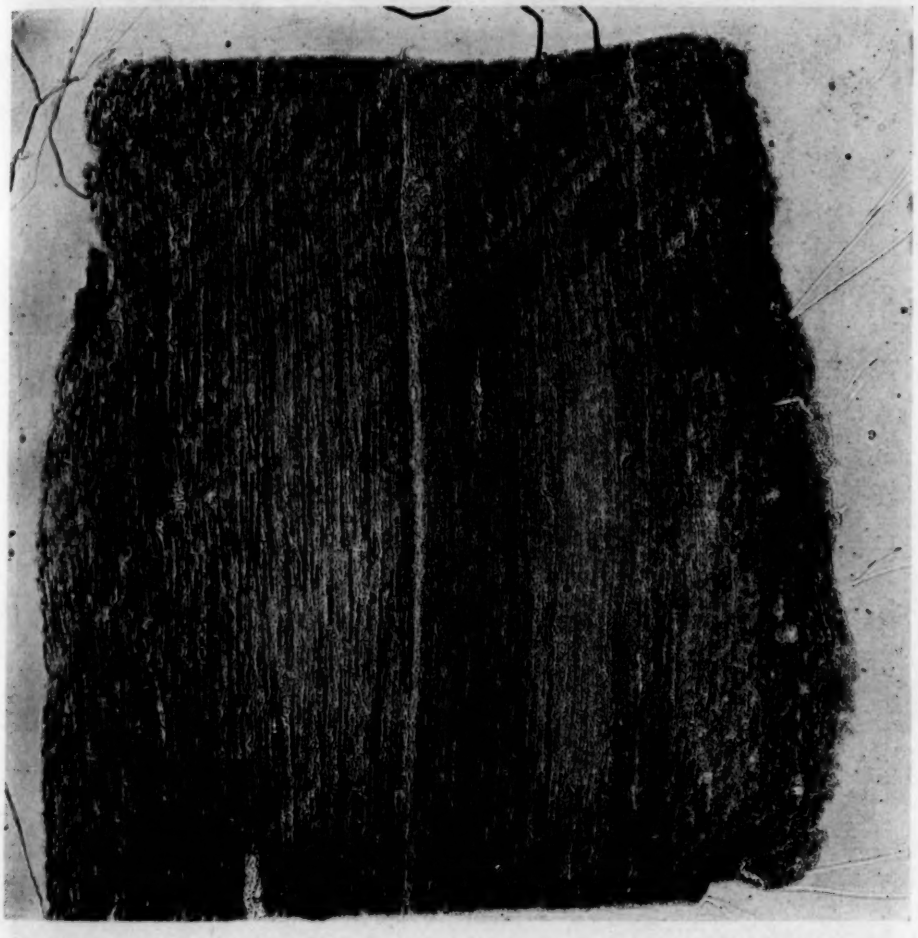

Fig. 1 (dog 9).—Photomicrograph of a longitudinal section from the spinal cord of a dog which lived for two months on the black tongue-producing diet and died showing stomatitis, glossitis, salivation, diarrhea, tremor and ataxia. The cord was fixed within one hour post mortem. The lesion is widespread and involves diffuse and irregular degeneration of myelin; much débris is present, especially along the margins of the section. Weigert-Pal stain; low power magnification (× 10).

observations. Some of the cords were cut in frozen sections and stained by the Spielmeyer method. These sections showed exactly the same condition of wide-

spread degeneration that appeared in the material stained by the Weigert technic. The cords stained by both the Weigert and the Spielmeyer method showed the lesions to be generally disposed around the margins of the sections, with the more central areas showing relatively little alteration. There was no perceptible differ-

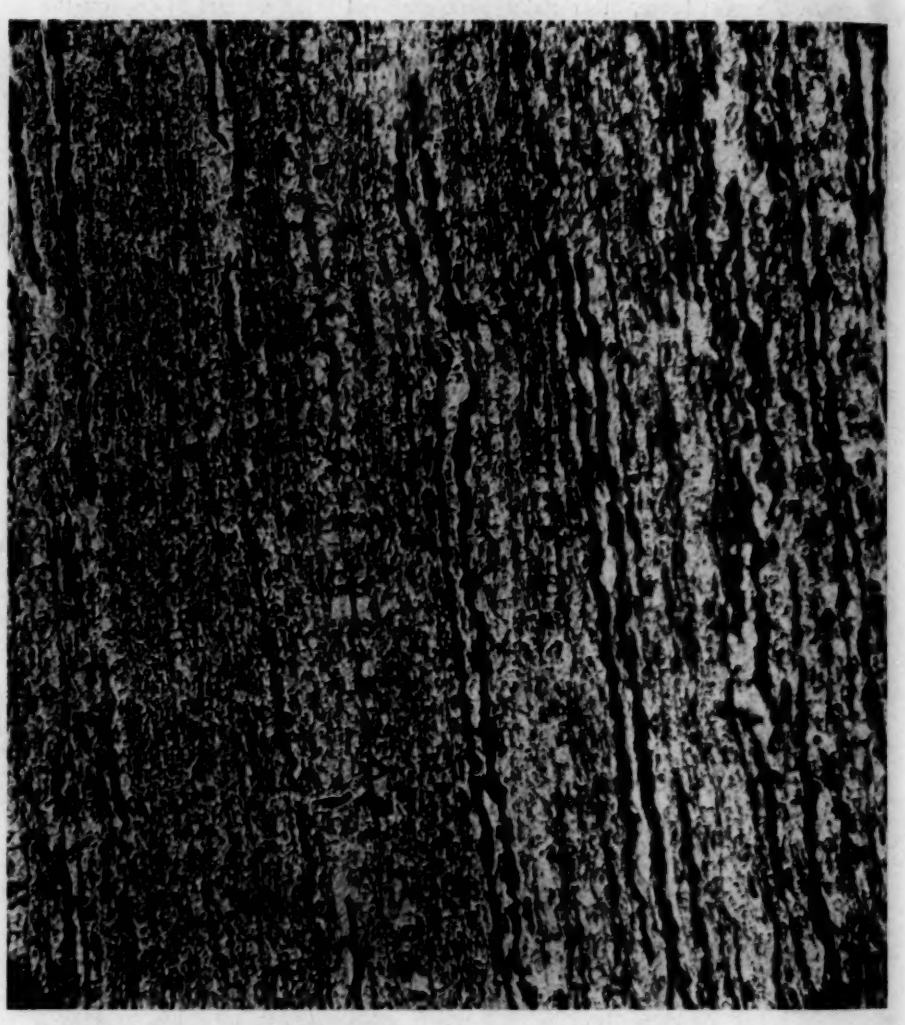

Fig. 2 (dog 4).—A longitudinal section from the spinal cord of a dog which lived for three and a half months on the black tongue-producing diet. At death the dog showed glossitis, stomatitis, diarrhea and tremor. The cord was fixed within one hour post mortem. The section shows widespread degeneration of the myelin, with few normal sheaths; there is much débris at the right of the section. Weigert-Pal stain; high power magnification (× 185).

ence between the sections from dogs the cords of which were fixed at once and those which may have been dead several hours before the autopsy.

Because the stains for fat showed no changes in the white matter, other attempts were made to determine the nature of the products of degeneration. Nile blue sulphate was used to stain any free fatty acids present, but none was demon-

Fig. 3 (dog 5).—Photomicrograph of cells in the anterior horn of a dog which lived three months on the black tongue-producing diet and died showing stomatitis, glossitis, salivation, diarrhea and tremor. The cord was fixed within one hour post mortem. The anterior horn cells show condensation of the Nissl substance and encrustations. Cresyl violet stain; immersion in oil; × 380.

strated. It was considered impossible to use Marchi's method because of the type of fixation employed. The technic described by Donaggio was used instead. This

method is stated to demonstrate very early myelin degeneration, staining the pathologic sheaths black in a colorless field. The picture thus produced is similar to that made by the Marchi method, but is said to give a positive stain of

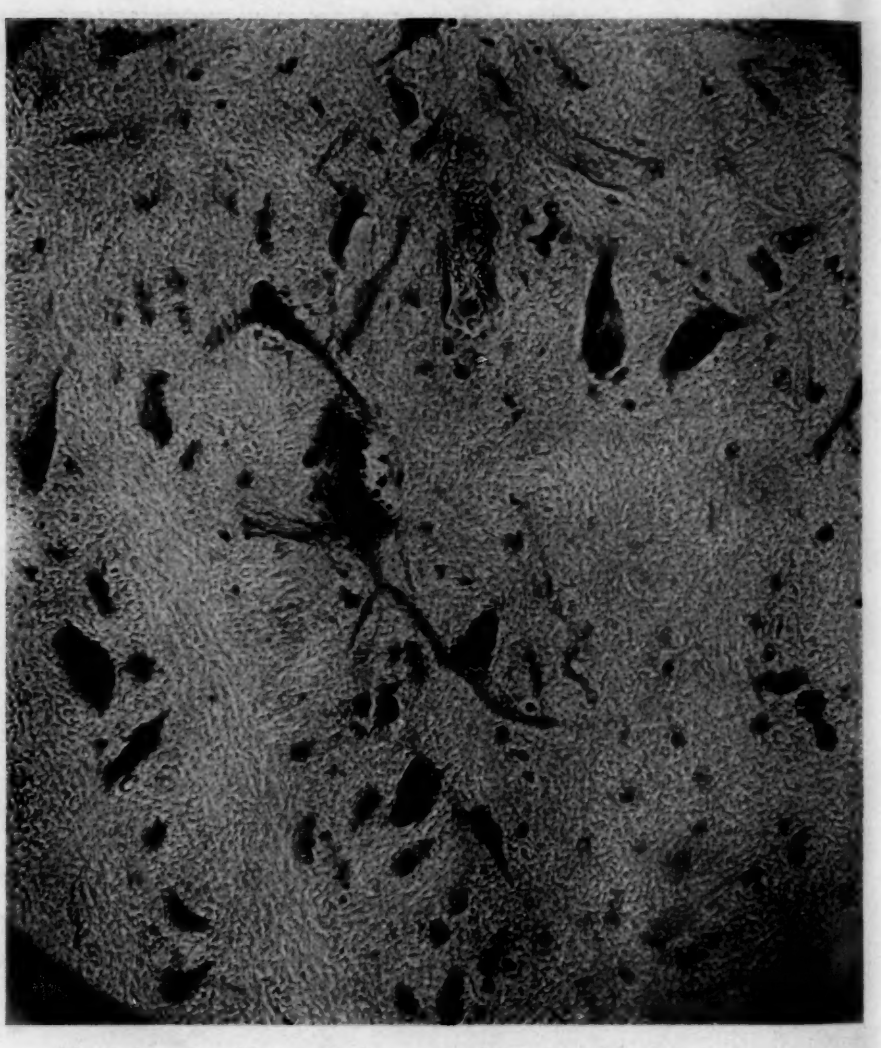

Fig. 4 (dog 3).—Nerve cells from a longitudinal section of the cord of a dog which lived for three and a half months on the black tongue diet and showed stomatitis, glossitis, salivation and diarrhea at death. The cord was fixed within one hour post mortem. The section shows atrophy and degeneration of the nerve cells; two cells in the left center show condensation of the Nissl substance and encrustations. Cresyl violet stain; immersion in oil; × 380.

degeneration within from three to five days. The material embedded in pyroxylin, which was prepared for staining by the Weigert method, was suitable for use

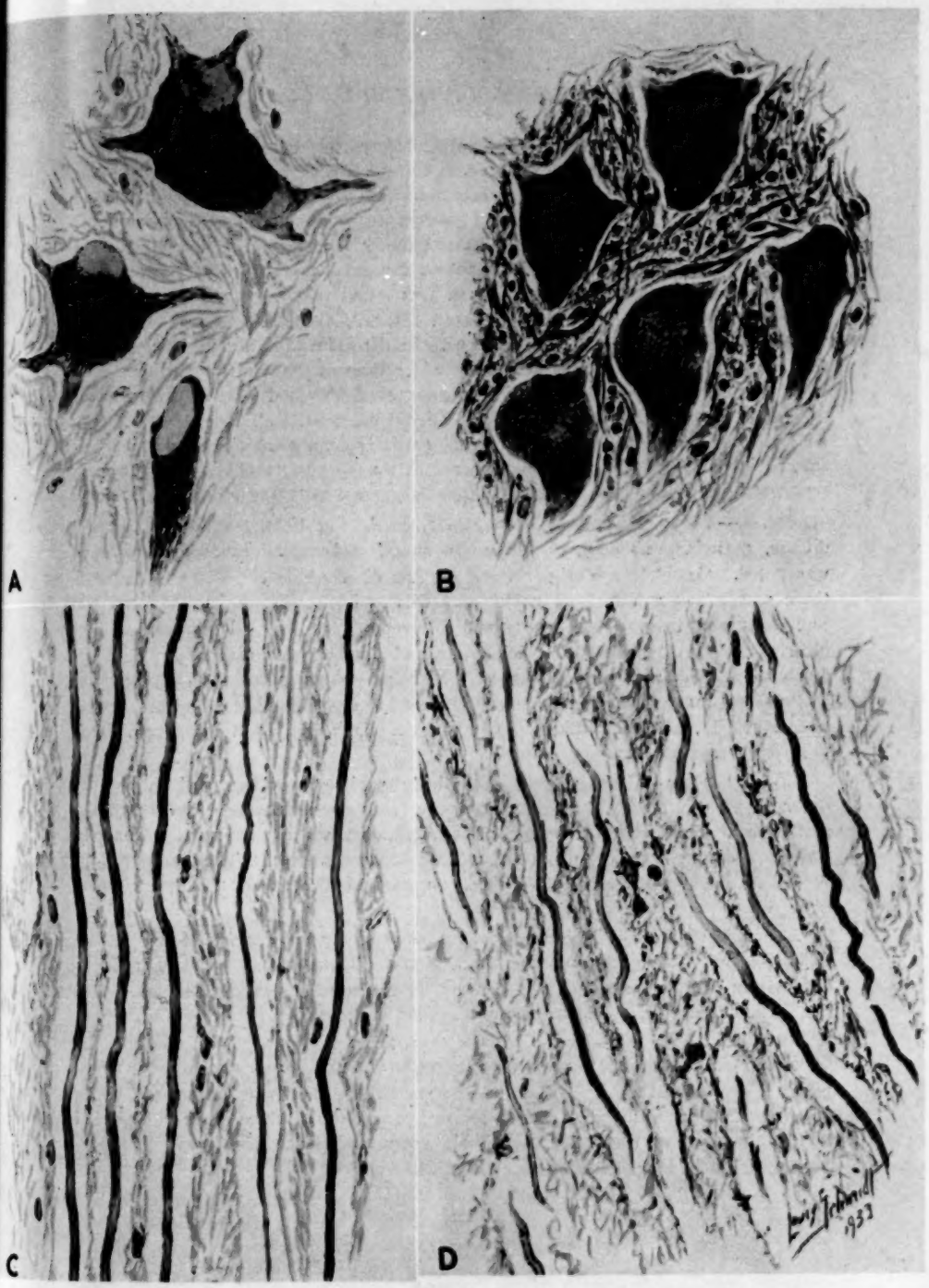

Fig. 5.—*A*, anterior horn cells from the cord of one of the dogs with black tongue, showing the large deposits of lipoid pigment, which stained green, with chromatolytic and lipoid changes in the nuclei; cresyl violet stain; reduced from × 450. *B*, anterior horn cells from one of the dogs with black tongue, showing the lipoid deposits, which stained red; Herxheimer scarlet red stain; reduced from × 450. *C*, longitudinal section of the cord of a normal dog, showing continuous axis-cylinders, which stained blue, and regularly arranged stroma; Alzheimer-Mann stain; reduced from × 450. *D*, longitudinal section from the cord of one of the dogs with black tongue, showing broken, slightly tortuous axis-cylinders, which appeared reddish on staining, and pale, edematous, irregular stroma; Alzheimer-Mann stain; reduced from × 450.

(having been mordanted in Müller's solution before embedding), and cross-sections were cut and stained with hematoxylin as for the Weigert method. The sections were immersed in neutral copper acetate for approximately thirty minutes and then differentiated with oxalic acid and potassium permanganate in the conventional Pal manner. In the description of this technic by Herxheimer [18] it is stated that the normal sheaths are uncolored, except on the extreme margins of the sections. In the controls observed in this study it was found that a narrow rim of black sheaths occurred along the margins of all sections, and this rim was somewhat variable in thickness in different sections. In the animals dying of black tongue this rim of black sheaths was invariably wider than in the controls and coincided well with the apparent location of the lesion as determined by the Weigert and Spielmeyer stains. Since the stain is relatively unknown and since it was impossible to examine a large series of spinal cords from normal animals, it is considered inadvisable to lay too much emphasis on the results obtained. They are reported here simply because they checked in general with the other observations.

Dog 4.—The diet was begun on Jan. 7, 1932. On February 24 the chronic, diffuse, granular reddening of the entire buccal and lingual mucous membranes was noted. Complete achlorhydria and chronic diarrhea developed. On March 1 there was a severe attack of acute stomatitis and glossitis, which persisted for four days and from which the animal recovered. On April 1 diffuse, chronic glossitis and stomatitis set in, which progressed steadily, with diarrhea. On April 10 tremors and weakness developed. Rice polishings vitamin B_1 concentrate (Block) was administered intravenously. The symptoms continued and progressed until April 18, when the animal was comatose. On April 19 it was found dead. Autopsy was performed immediately.

Weigert Stain.—Cross-sections showed the periphery of the cord to be less dense than the center. The tissue along the periphery was ragged and contained many clumps of degenerated myelin. The longitudinal sections appeared irregularly stained. Many clumps of myelin and much débris were present. The cord appeared to be definitely diseased, since almost no part of the sections had a normal appearance.

Herxheimer Stain.—The cross-sections showed a rare droplet of fat in the white matter. The amount was so small as not to be significant, and it probably was not in phagocytic cells. The nerve cells contained a considerable amount of fat. The longitudinal sections showed similar changes.

Toluidine Blue Stain.—In the cross-sections the meninges appeared normal. The white matter was normal, except for glia cells with hyperstaining granular cytoplasm. No infiltration or gliosis was apparent. In the gray matter the nerve cells were faded, vacuolated and degenerating. Many of them were surrounded by satellites, but, on the whole, satellitosis was not marked. The longitudinal sections differed in no particular from the cross-sections.

Alzheimer-Mann Stain.—Cross-sections showed the periphery to be much less dense than the center. There was no variation in staining of the axis-cylinders and no infiltration. The longitudinal sections were irregularly stained, particularly at the periphery. There was considerable variation in staining of the axis-cylinders from blue through brilliant red. Some infiltration was present, and phagocytes in small numbers were present in the tissue.

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18. Herxheimer, G.: Technik der pathologisch-histologischen Untersuchung, Wiesbaden, J. F. Bergmann, 1912, p. 321.

Donaggio Stain.—The sections were typically positive. There were aggregations of black spots around the periphery, more than were seen in the controls.

Summary.—Examination of the stained sections showed: results with Weigert and Alzheimer-Mann methods, +++; poliomyelopathy, ++.

Dog 9.—The diet was begun on Feb. 26, 1932. On April 4 a red, inflamed patch of mucous membrane appeared under the upper lip. This extended and became progressively worse until within a few days there was a mild, chronic injection of the entire lingual and buccal mucous membranes. Diarrhea set in and persisted until death. On April 19 severe stomatitis and glossitis were noted, with salivation, tremor and ataxia. On April 24 the animal was found dead. Autopsy was performed immediately.

Weigert Stain.—The cross-sections showed fading and loss of myelin, but degenerated myelin was not present. Longitudinal sections showed a striking lesion of myelin. There was complete loss of staining in the involved area, with beading, swelling of sheaths and discontinuity. Much débris was present.

Herxheimer Stain.—Cross-sections showed no fat. Longitudinal sections showed no fat in the white matter but small amounts in the nerve cells and glia cells of the gray matter.

Toluidine Blue Stain.—Cross-sections showed the meninges to be normal. The glia cytoplasm was deeply stained. The myelin and axis-cylinders had taken a pale blue stain. In the gray matter the nerve cells were undergoing every form of degeneration. Some were vacuolated, with the nuclei gone. Some were dark-staining or vitreous. The longitudinal sections were not different from the controls, except for some satellitosis.

Alzheimer-Mann Stain.—The cross-sections showed many unstained areas, especially around the periphery, but no alteration in the staining of the axis-cylinders was present. Longitudinal sections showed an unquestionable lesion of myelin. Little normal myelin remained. The axis-cylinders were fragmented. There was no infiltration.

Donaggio Stain.—No convincing change was observed.

Summary.—Examination of the stained sections revealed: results with the Weigert method, +++, and with the Alzheimer-Mann method, ++ poliomyelopathy, +.

Dog 12.—The diet was begun on March 9, 1932. Inability to secrete free hydrochloric acid developed. On April 3 stomatitis, glossitis and salivation set in, which continued with remissions and exacerbations for ten days. On April 17 tremor, ataxia, and weakness appeared; these became progressively worse. On April 21 the animal was found dead. Autopsy was performed immediately.

Weigert Stain.—Cross-sections showed no significant changes. The longitudinal sections showed striking changes; most of the myelin sheaths were intact, but there were areas of irregular staining, with clumps of degenerated myelin.

Herxheimer Stain.—Cross-sections showed a few droplets of fat in the perivascular spaces of the white matter. There was much fat in the nerve cells and neuroglia of the gray matter, and some in the perivascular spaces. Longitudinal sections showed droplets of fat in most of the perivascular spaces in the gray matter and in almost all the nerve cells. Fat was present in some neuroglia cells as well.

Toluidine Blue Stain.—Cross-sections showed the meninges to be normal. The white matter was normal, except for neuroglia cells with hyperstaining cytoplasm

and stained processes. The nerve cells were vacuolated and disintegrating; many contained greenish pigment. The Nissl bodies were small and dustlike. Satellitosis was not marked.

Alzheimer-Mann Stain.—Cross-sections showed a variation in the intensity of staining. The meninges were slightly thickened and engorged. The white matter showed some irregularity of staining, especially at the periphery. The longitudinal sections were not very different from the controls.

Donaggio Stain.—Every section showed blackened spots and sheaths around the periphery, spreading inward toward the gray matter.

Tabular Summary of Results with Various Methods of Staining *

Dog	1 Weigert Stain	2 Alzheimer-Mann Stain	3 Herxheimer Stain	4 Toluidine Blue Stain	5 Donaggio Stain
1	++	+	++	+	—
2	+	++	+	+	—
3	—	+	—	+	—
4	+++	+++	++	++	+
5	++	+++	+	+	+
6	++	+++	—	+	—
7	++	++	++	—	++
8	++	+	++	+	+
9	+++	++	+	+	—
10	+	+	++	+	—
11	++	+	+	+	—
12	+	—	+++	+	++

* In column 1, — indicates that the results were not different from those obtained by the same method in control animals; +, that there was fading of some myelin sheaths, i. e., a definite failure to take the hematoxylin stain; ++, that there was a more advanced degree of the same change; +++, ,that large areas of fading were present with *Markballen* and phagocytosis.

In column 2, — indicates that the picture was comparable to that shown by the controls; +, that a definite number of axis-cylinders took a red stain; ++, that a more extensive process was noted; +++, that a large proportion of the axis-cylinders were red and that the stroma was largely disrupted.

In column 3, — indicates that the sections were like the controls; +, that more fat was present in some nerve cells than in any control; ++, that an intermediate lesion was observed; +++, that fat was present in at least 80 per cent of the nerve cells and that a small amount was present in phagocytes in the white matter.

In column 4, — indicates that a section was like the controls; +, that the cytoplasm of the glia cells was stained and that various forms of degeneration were present in about 25 per cent of the nerve cells (these changes consisted of the axonal reaction, swelling of the cell, chromatolysis, vacuolation, the presence of a greenish pigment and nuclear changes consisting of eccentrically placed or absent nuclei); ++, that the changes just described were present in approximately 50 per cent of the nerve cells and that some of the nerve cells showed encrustations.

In column 5, — indicates a picture falling within the wide range of the normal controls; +, means that the band of myelin sheaths around the margin of the cross-section of the cord which stained black was wider than in any control; ++, that there was a wide band of black-stained marginal sheaths and also that black sheaths were present in areas approaching the central gray matter.

Summary.—Examination of the stained sections revealed: results with the Weigert method, +, and with the Alzheimer-Mann method, —; poliomyelopathy, ++.

GENERAL SUMMARY

In eleven of the twelve animals studied, lesions were demonstrated by the Weigert method of staining. The lesions were in general not striking and had apparently produced no definite reaction in either the neuroglia or the cells taking part in inflammatory processes. The cytoplasm of the neuroglia cells throughout the entire series had stained, and in some instances was quite granular. This may have been the beginning of a reaction on the part of these elements.

In all the myelin stains the structural change was, for the most part, slight and consisted of irregularity, swelling and shrinking of the fibers. Only in occasional sections was there actual breakdown into droplets, and the droplets were probably never phagocytosed (dog 6 may be an exception to this). There was no definite free fat present in the white matter of any dog, except possibly dog 12.

The Alzheimer-Mann stain was used here chiefly to determine the condition of the axis-cylinders, although it also stained the myelin and stroma. Of the twelve dogs, eleven showed alteration in the axis-cylinders, manifested by a reddish stain instead of the normal blue; the axis-cylinders that were present in the lesions were swollen, broken up and tortuous, staining from purple through brilliant red.

Poliomyelopathy, indicating a change in the nerve cells, occurred in eleven of the twelve dogs. It was characterized by various types of degeneration in the nerve cells and the presence of fat in the cells. In dog 3 poliomyelopathy was considered present, although fat was not demonstrated because the Nissl stain showed such marked changes.

The significance of the results obtained with the Donaggio method of staining is unclear. The control animals showed such a wide range of variation that sections from the dogs with black tongue had to be interpreted with great caution. Only sections showing a definite increase over the maximum change seen in the controls were reported positive.

Interpretation of data such as those presented is difficult. The changes described appear to be of an early acute type and perhaps autolytic or chemical. The most marked alterations present were of the myelin sheaths, which took the stain irregularly and showed various forms of a degenerative process. The other components of the central nervous system also showed consistent, though mild, alterations, which were in general considered to be pathologic. In brief, there was evidence of a general disturbance of the structure of the central nervous system.

The etiology of the changes described is obscure. The addition of rice polishings to the diet should have insured an adequate supply of antineuritic vitamin B_1. It is known that the symptoms associated with the pathologic lesions may be prevented by the addition to the diet of a variety of food substances. Many of these substances are known to be rich in the thermostabile factor (vitamin B_2 [G]) effective in promoting the growth of rats maintained on an otherwise adequate diet. Examples are meat, milk and yeast. A commercial yeast concentrate known to be poor in the growth-promoting factor is also effective as a prophylactic. Liver extract-Lilly is also effective in this regard. Lack of vitamin B_1 or B_2 (G) or both does not give rise to the clinical syndrome described. It appears, therefore, that the condition is due to lack of an essential food substance which is as yet unidentified but which appears to be frequently associated with vitamin B_2 (G).

Comparison of the histologic alterations described in the protocols with those which have been found in the central nervous systems of human beings dying of pellagra shows a striking similarity. In view of the like pathology of other organs, the similar symptoms and the almost identical effective preventive and therapeutic measures, it seems likely that canine black tongue and human pellagra may be related etiologically. It appears that the deficiency which is causative in these two conditions gives rise to fairly characteristic lesions of the central nervous system, even though symptoms referable to that system may be slight and the disease process apparently of brief duration.

CONCLUSIONS

1. Lesions of the central nervous system are present in animals dying of acute black tongue.

2. The lesions are characterized by disintegration of myelin, alterations of the axons and degenerative changes of nerve cells.

3. The changes resemble those described in pellagra and in animals deficient in the vitamin B complex.

STUDIES IN ATHEROSCLEROSIS: CHEMICAL, EXPERIMENTAL AND MORPHOLOGIC

I AND II. RÔLES OF CHOLESTEROL METABOLISM, BLOOD PRESSURE AND STRUCTURE OF THE AORTA; THE FAT ANGLE OF THE AORTA (F.A.A.), AND THE INFILTRATION-EXPRESSION THEORY OF LIPOID DEPOSIT

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INTRODUCTION

Concerning the pathogenesis of atherosclerosis of the aorta there have been three important theories, viz., that it is infectious, degenerative or metabolic.

The first theory was championed by Virchow who believed the process to be partially infectious, and he spoke of an "Endarteritis chronica nodosa deformans." His idea was that the infectious process so altered the wall of the vessel that imbibition of fat from the serum of the blood stream followed and fat was deposited there. His theory in modified form still has some adherents (Klotz,[b] Saltykow *) but more generally has been discredited.

The infectious theory was then replaced by the degenerative one (Marchand, Jores, Thomas, Aschoff [c, d, e]), and here again two different stands were taken. Thoma's concept was that the media underwent a primary degeneration or weakening (*angiomalacia*) followed by compensatory proliferation of the intima (*Angiosklerose*) (see also Faber, Adami and Krauss, Beitzke, cited by E. Kaufmann). Marchand and Jores found no microscopic changes in the media of the aorta and ascribed the primary cause of atherosclerosis to a fatty degeneration of the intima. Thus, Marchand changed Lobstein's terminology of arteriosclerosis to that of atherosclerosis. The source of the fat of the intima was then shown by Aschoff not to be a degeneration of the intima but rather an infiltration from the nutrient blood plasma ushered in from the lumen of the aorta, thereby reviving Virchow's original imbibition theory (Ribbert, Stumpf, Hueck, cited by Jores).

The cause of the primary degenerative process was considered to be the outcome of the excessive functional demand made on the aorta (Thoma, Marchand, Beneke, Moschcowitz, Lange,[b] Rühl) or the conse-

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* The bibliographic references will be found at the end of the fourth article, which will be published in the November issue.

From the Pathologic Institute, Freiburg, Germany; Dr. L. Aschoff, director; the Pathologic Institute of the Cook County Hospital, Chicago; Dr. R. H. Jaffé, director, and the chemical division of the Pathologic Institute, Freiburg, Germany; Dr. R. Schoenheimer, formerly director.

quence of the wear and tear of age (Romberg,[b] Wells). Accordingly, the entire process of atherosclerosis was set down as inevitable.

The metabolic trend of thought takes its initiative from Aschoff,[a, b] who showed that the doubly refractive bodies in atheromatous aortas were similar to cholesterol esters, and that they infiltrated from the blood stream; and its challenged verification, from Anitschkow and Chalatow, also Wacker and Hueck, who produced atheromatous lesions in the rabbit's aorta by feeding cholesterol.

Although each school of thought at its inception believed the evidence which it presented was the primary and sole cause of the atherosclerosis, in the later development all in turn have conceded that the secondary factors were of utmost importance. What is primary and what is secondary in the evolution of atherosclerosis has not been definitely proved, but it is encouraging to know that there are several components and that some of these may be influenced.

Accrued from present knowledge, atherosclerosis depends on the following constituents:

1. Cholesterol metabolism
2. Physical and chemical changes of the aortic wall as influenced by the blood pressure, age and intoxication
3. Constitutional or hereditary disposition

Which of the foregoing factors are primary and which are secondary, which can be influenced and which cannot be, are questions that must be explored before any hope of the alleviation of atherosclerosis can be entertained.

With the pendulum swinging toward the belief that atherosclerosis depends on cholesterol metabolism, this possibility in the pathogenesis should be exploited, as it is a factor that may be partly controlled.

The scope of this work embraces only atherosclerosis of the aorta and the immediate factors that influence it. What determine the latter will not receive serious consideration.

The infectious type of lesion (syphilis and rheumatic fever), calcification of the media of the muscular arteries (Mönckeberg's sclerosis), periarteritis nodosa, necrosis of the media (Erdheim), arteritis obliterans, involution sclerosis (ductus Botalli, umbilical cord, vessels of the female organs) and pure senile sclerosis have ostensibly a dissimilar etiology (Jores and Kaufmann) and will not be discussed.

I. PRELIMINARY DETERMINATIONS

A. THE FAT CONTENT OF THE AORTA IN 500 CASES OF THE WHITE AND THE COLORED RACE

Because recent experimental, pathologic and clinical observations have indicated a possibility that the cholesterol metabolism may influence the development of atherosclerosis of the aorta, every effort should

be made to verify or disprove this assumption, for the universally accepted notion is that this process is inevitable and the consequence of senescence.

The literature contains a multitude of reports correlating atherosclerosis with diet, blood pressure, infections, environment, climate, race, etc. These communications have in many instances been contradictory because of the lack of uniformity in the methods of examination and also because of the diversity of source.

In the majority of the accounts the diagnosis of atherosclerosis is based on the blood pressure and clinical examinations. That hypertension predisposes to atherosclerosis is granted, but the two conditions are not synonymous and may exist independently. The diagnosis of atherosclerosis of the aorta by examination of the peripheral vessels (rolling them between one's fingers—Stocks) is misleading, as the association of atherosclerosis of the aorta with atherosclerosis of the peripheral vessels in the early stages of the disease is uncommon. Indeed, when the peripheral vessels are affected, the aorta is usually spared (Jores). With present methods of examination the diagnosis of atherosclerosis of the aorta is impossible except when the condition is far advanced (x-ray pictures and Lange's capillary test).

The nonconformity of the statistics have confused what little there was known, and many avenues of research have been discontinued and the workers discouraged because of this. That an application of experimental data to man can be reached only through statistical studies is easily understood. Such information can be accepted only if the investigators conform to a standard method of examination.

As clinical methods for the early recognition of atherosclerosis are still unsatisfactory, it follows that postmortem evidence is necessary. But again difficulties arise in that different pathologists evince varying opinions as to the severity of atherosclerosis. What is considered as severe atherosclerosis in Freiburg, e. g., is adjudged as moderate to slight atherosclerosis in Chicago. Moreover, diffuse atherosclerosis, whether focal or generalized, may be overlooked if the intima is not appreciably altered grossly.

Standard criteria were consequently left to be desired until Aschoff noted that the doubly refractive bodies in atheromatous aortas might be cholesterol esters, and he proved it chemically through the work of Windaus. Unfortunately, the detailed results of the latter cannot be accepted because he failed to remove the adventitia of the aorta and thus included large quantities of fat that was not related to the disease process. It was Schönheimer who realized this error and who by repeating the work of Windaus, removing the adventitia beforehand, found that the total amount of extractable fat in the aorta was directly proportional to the degree of atherosclerosis. Moreover, the lipoid was

mainly composed of free cholesterol and its ester, and in atherosclerotic aortas the relationship between these elements was constant, viz., 25 and 57 per cent, respectively.

The analyses of Schönheimer, because of their extensive chemical nature, cannot be used as a routine procedure. But as the proportions of the fatty constituents remain constant (in atherosclerosis) a determination of the total fat content should suffice. The elaboration of this principle was suggested as a problem for research by Dr. Schönheimer.

The material consisted of 500 aortas of adults over 25 years of age, male and female, of the white and the colored race. The autopsies were performed at the Cook County Hospital in Chicago, and the analyses were carried out in the chemical division of the Pathologic Institute in Freiburg, Germany.

Method.—The aorta was cut as close to the valves as possible and at the bifurcation of the aorta. All the branches were cut flush to the surface of the aorta. With a pair of forceps, or better still, with the finger-nails, the adventitia was separated at one point from the media, the line of cleavage being found. Once this separation was brought about, the stripping was simple, as the adventitia, especially in atheromatous aortas, separated very readily from the media. Small muscle fibers of the media remained attached to the adventitia, but the amount of fat contained therein is negligible (Schönheimer [c, d]).

The aorta was then chopped in very fine bits by means of a meat chopper or a specially constructed semicircular blade about 20 cm. in diameter with wooden handles on both ends. A hard wooden board with elevated border was used to prevent the loss of any of the material. The finely chopped mass was then placed in a mortar and ground to a fine dry powder with a minimal amount of anhydrous sodium sulphate.[1] The dried aorta was placed in an extraction bottle and a measured amount of carbon tetrachloride (double-distilled) was added so that the fluid extended at least 1 cm. above the solid matter after a thorough shaking. It was safer to use more extraction fluid than less. The extraction was allowed to continue for from four to twelve hours with intermediary shakings every fifteen to thirty minutes. The fat-containing fluid was filtered off, and in a previously weighed evaporating dish a measured quantity of this fluid was evaporated on a water bath. After complete evaporation, the dish was placed in an electric oven at 120 C. for one hour. The dish was weighed again after it had been allowed to cool in a desiccator. The process of drying and weighing was continued until the weight was constant. The amount of fluid used as solvent being known, as well as the amount evaporated and the weight of fat contained in the latter, the total fat content of the aorta was accordingly determined.

Results.—Table 1 represents the average amount of fat in the aorta for each of the age groups indicated. Included in this table are both

1. As the material was gathered in Chicago and the extractions made in Freiburg, Germany, it was necessary after drying with sodium sulphate to remove all moisture in a desiccator. The dried substance was wrapped in ether-extracted cotton cloths and preserved in sealed tin cans on ice. These cans, hermetically sealed, were shipped to Europe. The aortas remained perfectly dry and showed no sign of deterioration.

races and sexes. A progressive increase of fat with age is noted, and when these values were plotted (fig. 1*a*) the curve thus formed approached almost a straight line. The slight deviations could be accounted for by possibilities of error due especially to incomplete extraction.[2]

The heterogeneousness of the group prevented generalization, and in table 2 the two races were separated. Here again an increased fat content of the aorta was noted with age for both races. Between 25 and

TABLE 1.—*The Average Amounts of Fat in the Aorta for All Cases*

Age	Cases	Fat, Gm.	Age	Cases	Fat, Gm.
25–30	42	0.106	51–60	98	0.503
31–40	86	0.185	61–70	67	0.833
41–50	117	0.355	71–	45	1.162

TABLE 2.—*The Average Amounts of Fat in the Aorta for Each Race*

	White		Colored	
Age	Cases	Fat, Gm.	Cases	Fat, Gm.
25–30	15	0.064	27	0.128
31–40	44	0.155	42	0.218
41–50	81	0.366	36	0.372
51–60	13	0.518	25	0.485
61–70	57	0.831	10	0.845
71–	38	1.122	7	1.382

TABLE 3.—*The Average Amounts of Fat in the Aorta for All Cases in Each Sex of Both Races*

	White				Colored			
	Male		Female		Male		Female	
Age	Cases	Fat, Gm.	Cases	Fat, Gm.	Cases	Fat, Gm.	Cases	Fat, Gm.
25–30	5	0.077	10	0.053	8	0.120	19	0.132
31–40	24	0.187	20	0.117	26	0.214	16	0.222
41–50	52	0.351	29	0.366	21	0.219*	15	0.541
51–60	52	0.511	21	0.535	15	0.424†	10	0.518
61–70	40	0.805	17	0.893	8	0.995	4	1.036
71–	25	1.086	13	1.189	5	1.289		

* When syphilitic aortitis with atherosclerosis was included—0.266 Gm.
† When syphilitic aortitis with atherosclerosis was included—0.467 Gm.

40 years the amount was greater for the colored race. The plotted values (fig. 1*b* and *c*) formed curves similar to each other and to that of the total group.

Further division according to sex (table 3) revealed that the white male presented higher values than the white female up to 40 years, at

2. Schönheimer allowed for an error of 10 per cent under 0.06 Gm. of fat and an error of 4 per cent over 0.1 Gm. However, he extracted his aorta for one hundred and forty-four hours. The percentage of error for each individual case in the present work would be much greater, as the extraction was carried out only for from four to twelve hours. Considering the large number of cases employed, the sum total of error naturally becomes much diminished.

which time the latter surpassed the former. In the colored race the female superseded the male throughout when sufficient cases were available. In comparing the two races, the colored male on the average had more fat in his aorta than the white male, except between the ages of 41 and 60 years. This was accountable for by the fact that syphilitic

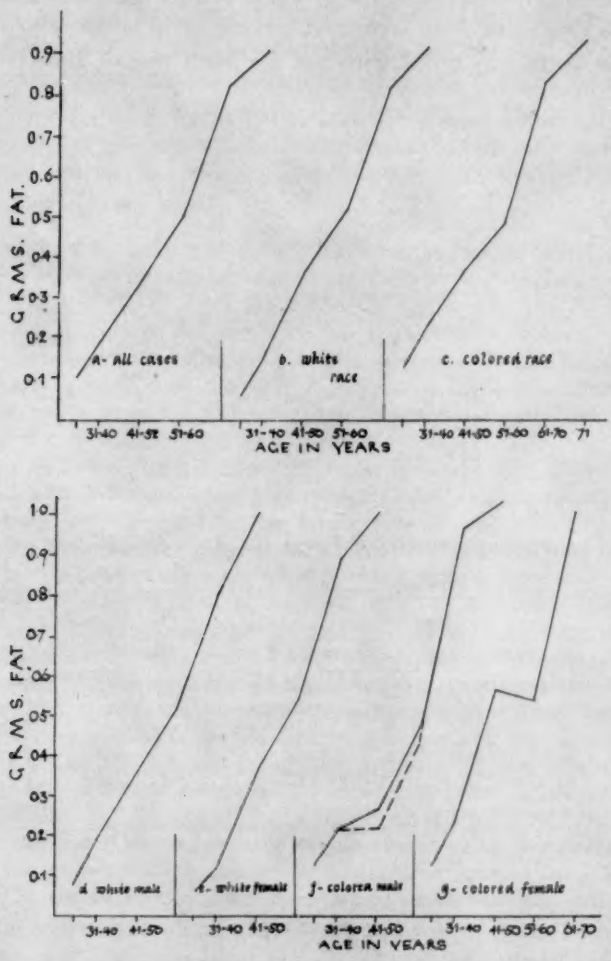

Fig. 1.—Curves of the average amount of fat in the aorta in different age groups of both races and of each race and of the two sexes of each race.

aortitis, whenever present, was excluded in the foregoing analyses. As the colored male at the Cook County Hospital has a higher incidence of syphilitic aortitis (22.02 per cent—Jaffé [a]) as compared with the white male (7.43 per cent—Jaffé), the exclusion of these cases naturally eliminated cases with atherosclerosis. When such aortas were included the average fat values rose.

The colored female in general showed a higher fat content of the aorta than the white female (more detailed and error-corrected comparisons will be given in part C).

Figure 1 *d, e, f* and *g* tenders the curves for the sexes in both races. For the white male and female, whose averages embraced the largest number of cases, the curves approached straight lines. For the colored male a wide bowing was noted between 41 and 60 years and was accounted for by the exclusion of cases with syphilitic aortitis.[3] The insufficient number of cases of colored females accounted for the irregularity of the curve.

Summary.—The amount of fat in the aorta increased with age, and when a sufficient number of cases in a homogeneous group were available, the ascent approached a straight line.

B. THE RELATIONSHIP BETWEEN THE QUANTITY OF FAT IN THE AORTA AND ATHEROSCLEROSIS

As has been stated in the previous part, the work of Aschoff, Windaus and Schönheimer strongly suggested that the amount of fat in the aorta was parallel to the degree of atherosclerosis. Because their work was carried out on a limited scale, no far-reaching conclusions could be made.

It is necessary to establish definitely such a relationship, as knowledge of the fat content of the aorta would mean little if it did not have a direct bearing on atherosclerosis.

In order to determine this relationship a set of tables was constructed in which the fat contents of the aortas of individuals were subdivided according to sex, age, race and severity of atherosclerosis from a macroscopic standpoint. It may be added at this point that the macroscopic examinations were made close at hand before the aortas were prepared, and all changes were noted. In this preliminary table, three divisions were made, viz., "smooth aortas," "aortas showing slight to moderate atherosclerosis" and "aortas showing moderate to severe atherosclerosis."

Under "smooth" were considered all aortas in which the intima was practically smooth and included small yellow stripes and plaques in almost all cases. Under "slight to moderate atherosclerosis" were included aortas showing discrete node formation of atheromatous or hyaline character and an occasional calcific plaque or ulceration. "Moderate to severe atherosclerosis" embraced all the remaining aortas.

3. Not all cases with syphilitic aortitis were analyzed, but with the addition of a portion of these cases, the curve does not approach that of the white male. This tends to show that syphilitic aortitis and atherosclerosis of the aorta are not related.

In this table many discrepancies were found which could be explained by two possibilities: First, aortas showing local or diffuse atherosclerosis, in which intimal changes are often very slight, may have been included under "smooth aortas." This was particularly common in malignant and, to a less extent, benign hypertension and nephritis. On the other hand, senile ectasia was sometimes misleading and interpreted as severe atherosclerosis. Second, the borderline cases were exceedingly difficult to separate.

To overcome both of these difficulties in part, the mean high and low values were determined for each age group, sex and race, and a new set of tables was constructed in which only these mean fat values were considered, no consideration being made of the gross description. Under this division there were very few discrepancies, although the average fat values for each group remained unchanged. Thus one has a table interpreting atherosclerosis in terms of the fat contents, although the gross description formed the basis of this division.

TABLE 4.—*Fat Values for Smooth Aortas*

		White				Colored			
		Male		Female		Male		Female	
Age	Fat Limits	Cases	Fat, Gm.	Cases	Fat, Gm.	Cases	Fat, Gm.	Cases	Fat, Gm.
25–30	0.02–0.15	5	0.077	10	0.059	7	0.057	14	0.092
31–40	0.06–0.15	15	0.081	17	0.079	15	0.093	9	0.092
41–50	0.06–0.19	26	0.139	10	0.131	14	0.117	4	0.099
51–60	0.06–0.21	14	0.152	7	0.132	4	0.138	2	0.112
61–70	0.06–0.22	3	0.182			2	0.197	1	0.164
71–									

Of course, the interpretation of the intensity of the atherosclerosis was an individual interpretation and might not coincide with that of other authors. But, as the fat content and the severity of the atherosclerosis of the aortas corresponded so closely, this table may serve as a standard of comparison with values from other localities.

In the 179 cases considered under "smooth aortas" (from 0.02 to 0.22 Gm. of fat according to age) there were 6 discrepancies, an error of 3.3 per cent (table 4). These cases showed grossly an occasional nodule and in no instance an outspoken atherosclerosis. When it is considered that 5 of these cases were in persons aged 48 years and over, the possibility that the alterations were senile or that the fat might have been replaced by hyalin is great. In any case, the percentage of error is practically negligible.

Of the 170 cases of atherosclerosis classified as "moderate to severe" (0.16 to 0.8 Gm. of fat according to age—table 5) there were 17 in which the gross description corresponded to "smooth," a 10 per cent discrepancy. These aortas were found in cases of malignant or benign

hypertension and of nephritis. The later was the result of a diffuse atherosclerosis being overlooked on gross examination.

Under "moderate to severe atherosclerosis" (over 0.45 Gm. of fat—table 6) there were no cases in which gross evidence of atherosclerosis was lacking.

The discriminations between the borderlines of slight to moderate and moderate to severe atherosclerosis were more difficult to ascertain, as a definite distinction was not possible. For this reason allowance was made for overlapping: thus the separation of slight to moderate

TABLE 5.—*Fat Values for Slight to Moderate Atherosclerosis of the Aorta*

		White				Colored			
		Male		Female		Male		Female	
Age	Fat Limits	Cases	Fat, Gm.	Cases	Fat, Gm.	Cases	Fat, Gm.	Cases	Fat, Gm.
25–30	0.16–0.44							4	0.215
31–40	0.16–0.44	6	0.213	2	0.169	8	0.269	6	0.338
41–50	0.20–0.5	20	0.340	14	0.325	11	0.305	3	0.295
51–60	0.22–0.6 incl.	26	0.440	40	0.439	10	0.503	5	0.381
61–70	0.22–0.7 incl.	20	0.497	9	0.556	4	0.648	1	0.626
71–	0.23–0.8 incl.	11	0.575	5	0.674	2	0.654		

TABLE 6.—*Fat Values for Moderate to Severe Atherosclerosis of the Aorta*

		White				Colored			
		Male		Female		Male		Female	
Age	Fat Limits	Cases	Fat, Gm.	Cases	Fat, Gm.	Cases	Fat, Gm.	Cases	Fat, Gm.
25–30	0.45 and over					1	0.557	1	0.538
31–40	0.45 and over	3	0.606	1	0.659	3	0.674	1	0.691
41–50	0.5 and over	6	1.308	5	0.951	2	0.716	8	0.855
51–60	0.7 and over	12	1.082	4	1.480	1	0.778	3	1.017
61–70	0.8 and over	17	1.277	8	1.268	2	2.324		
71–	0.9 and over	14	1.486	8	1.511	3	1.718	2	1.614

and moderate to severe. There were 27 cases in the middle group that were grossly interpreted as moderate or severe atherosclerosis, whereas there were but 6 cases in the last group in which a slight to moderate atherosclerosis was present.

Taken all in all, the foregoing classification seemed justified. The percentage of error between the smooth and the atheromatous aortas was almost negligible. It was, after all, this differentiation that was important.

It was of interest to note that in the group of severe atherosclerosis the incidence of ulceration over 41 years was 68 out of 93, or 73 per cent. Yet, in spite of the loss of fat that certainly took place with ulceration, its bearing on the total amount was negligible and this answers possible objections to the classification of atherosclerosis accord-

ing to the fat content of the aorta. Under 40 years the number of cases were too few to allow one to make any generalizations.

Calcification, when severe, may also replace some of the fat, but in a large group of cases this error may be disregarded.

Tables 4 to 6 give the average amounts of fat of the aorta according to the method that has been specified, and it will be noted that in the smooth aortas the amounts of fat were practically similar for both races and sexes. The slight differences could be easily explained by the possibilities of error.

In order to establish a larger number of cases for comparison the fat values of the slight to moderate and moderate to severe atherosclerosis were combined (table 7). In atherosclerosis, the white female up to 50 years of age had a lesser tendency toward fat deposit in the aorta than the white male, and after 50 years she slightly surpassed him. For the colored race the inclination to fat deposit of the female was

TABLE 7.—*Fat Values of All Cases of Atherosclerosis of the Aorta*

		White				Colored			
		Male		Female		Male		Female	
Age	Fat Limits	Cases	Fat, Gm.	Cases	Fat, Gm.	Cases	Fat, Gm.	Cases	Fat, Gm.
25–30	0.16 and over					1	0.557	5	0.280
31–40	0.16 and over	9	0.363	3	0.332	11	0.339	7	0.389
41–50	0.20 and over	26	0.563	19	0.484	10	0.474	11	0.702
51–60	0.22 and over	38	0.643	14	0.736	16	0.550	8	0.619
61–70	0.22 and over	37	0.856	17	0.893	6	1.207	1	0.626
71–	0.23 and over	25	1.086	13	1.189	5	1.289	2	1.614

always greater than that of the male (when sufficient cases were had for comparison). The colored race had an appreciable amount of fat in their aortas from 25 to 30 years, much higher than that of the white race. The colored female presented the highest values up to 50 years of age (cf. part C for complete comparisons).

In a large group of cases, as has been shown, the amount of fat in the aorta ran parallel to the degree of atherosclerosis. These findings verify the suggestions of Schönheimer, whose work was carried out on a small scale. One has then a simple method of comparison that can be used the world over.

What are the advantages of this method over the macroscopic and the microscopic one? From a macroscopic standpoint it is impossible to measure the amount of atherosclerosis as one does an aneurysm, for example. Further, few atherosclerotic plaques in the aorta of an old person might pass as normal and the condition be recorded as a nonatherosclerotic state (considering his age). On the other hand, discrete plaques in a young person might be considered as atherosclerotic. Finally, not all pathologists show the same interest in certain pathologic

states. Considering, then, that most statistics are compiled as afterthoughts, the discrepancies are great.

Microscopic sections are also misleading when the aorta is considered as a whole, as sections are invariably made of the affected areas. Serial sections of the aorta are out of the question when considered as a routine procedure. On the other hand, the simple chemical procedure described gives one a complete picture at once and, in certain respects, is far superior to the serial sections. In the former, one is dealing with a numerical quantity that can be compared with mathematical precision while in the latter, one is dependent on the interpretations of the observer. It is only by adopting such a method that accurate scientific comparisons can be made.

Comment and Summary.—The fat content of the aorta, although increasing with age, was found to be directly proportional also to the severity of the atherosclerosis. The facts that the fat deposit was more intense in the young adults of the colored race and that there were variations with sex speak against age as the only determining factor. Because the correlation between the fat content and the atherosclerosis of the aorta in a large group of cases is so precise, it is suggested that the former is not merely a sequence of the latter but that the two are closely dependent on each other. In other parts of the body, e. g., degenerative processes, fibrosis and hyalinization are not accompanied by a corresponding deposit of fat.

C. THE INCLINATION TO ATHEROSCLEROSIS OF THE AORTA AS DETERMINED BY THE FAT ANGLE OF THE AORTA (F.A.A.)

In figure 1 *d* and *e*, in which a large homogeneous group came under consideration, the increase of fat in the aorta followed an almost straight course. It was conjectured that if the angle of this inclination could be measured, a simple method of comparing atherosclerosis of the aorta would be had. In order to further this hypothesis, a common scale was adopted so that comparisons could be made with other authors. The scale chosen was similar to that employed in plotting the values in figure 1, as the angles thus formed were of appreciable size and easily measurable. In this graph each millimeter was considered as 0.01 Gm. of fat and also as 1 year. The calculation of an angle may be made by the formula:

$$\text{tangent of an angle} = \frac{\text{altitude}}{\text{base}}$$

in which the altitude is equal to the gram of fat and the base to the age in years. A correction of this formula was necessary because the fat values between 25 and 30 years may vary so that a fixed point was placed at 15 years at which time the fat content is usually constant (about

0.2 Gm.—Schönheimer). Atherosclerosis if present at this age is a rarity. The complete formula is

$$\text{tangent of the angle} = \frac{(\text{grams of fat in the aorta} \times 100) - 2}{\text{age} - 15}$$

With this formula the angles were determined for the various age groups (the groups were considered as a whole, since the larger the group the less was the possibility of error). The mean of all the angles was designated, and this value was considered as the "fat angle of the aorta" (F.A.A.), or the inclination to atherosclerosis of the entire group. The advantage of this method of calculation is that it embraces at once the severity of the atherosclerosis and the incidence.

TABLE 8.—*The Fat Angle of the Aorta (F.A.A.) for All Cases*

Age	Fat, Gm.	F. A. A., Degrees
25–30	0.106	34.3
31–40	0.185	39.6
41–50	0.355	48.2
51–60	0.503	51.4
61–70	0.833	58.4
71–	1.102	62.4
Average F. A. A.		49.1

TABLE 9.—*The Fat Angle of the Aorta (F.A.A.) for Each Race*

	White		Colored	
Age	Fat, Gm.	F. A. A., Degrees	Fat, Gm.	F. A. A., Degrees
25–30	0.064	19.6	0.128	40.8
31–40	0.155	34.0	0.217	44.8
41–50	0.357	48.4	0.372	49.5
51–60	0.518	51.2	0.485	49.4
61–70	0.831	58.4	0.845	58.8
71–	1.122	61.4	1.382	66.5
	Average F. A. A.	45.5	Average F. A. A.	51.6

The fat angle of the aorta (F.A.A.) or the inclination to atherosclerosis of the entire group of cases studied was disclosed to be 49.1° (table 8).

The foregoing value applies to the poorest class of people living in Chicago under similar economic conditions for a greater or a lesser number of years. Of the white persons a high percentage of the older adults were foreign-born (Germans, Irish, Italians, Poles, Croats) but had lived in Chicago from prewar times. A later influx has been guarded against by the strict immigration laws. Among the colored persons practically all were southern-born and the duration of their residence in Chicago was as a rule proportional to their age (from 25 to 31 years—six years: from 71 to 80—forty years).

Comparing the two races (table 9), the colored persons in the second to fourth decades of life had a greater inclination to atherosclerosis

than the white persons. From 41 years onward the two races showed no appreciable differences until after 71 years, when the colored race again had a predominant degree of atherosclerosis. These differences were far beyond the margin of error, as between 25 and 30 years the difference was 100 per cent and between 31 and 40 years and over 71 years it was about 25 per cent.

The average fat angle of the aorta (F.A.A.) for the white race was somewhat lower (45.5°) than that for the colored race (51.6°). Although this difference falls within an error of 15 per cent and may be discounted, the individual group differences were such that one was justified in saying that the colored race had a stronger inclination to atherosclerosis than the white race. The contention of Stocks is similar to this, but he based his conclusions mainly on clinical diagnosis (death

TABLE 10.—*The Fat Angle of the Aorta (F.A.A.) for Each Sex in Each Race*

	White				Colored			
	Male		Female		Male		Female	
Age	Fat, Gm.	F. A. A. Degrees	Fat, Gm.	F. A. A., Degrees	Fat, Gm.	F. A. A., Degrees	Fat, Gm.	F. A. A., Degrees
25–30	0.077	22.9	0.053	14.8	0.120	38.6	0.132	44.3
31–40	0.187	39.8	0.117	25.9	0.214	44.2	0.222	47.4
41–50	0.351	48.5	0.366	49	0.266	39.5	0.542	60.4
51–60	0.501	50.8	0.535	52.8	0.468	48.2	0.518	50.8
61–70	0.805	57.6	0.893	60.2	0.955	61.8	1.006	61.4
71–	1.086	60.5	1.189	62.9	1.289	64.8		
Average F. A. A.....		46.7		44.4		49.5		52.8

certificate statistics). Camac, who is frequently quoted in relation to atherosclerosis of the colored race, reported only on syphilitic aortitis.

A comparison of the sexes (table 10) revealed that the white female had a lesser tendency to atherosclerosis than the white male at the age of from 25 to 40 years, but that after 40 years the difference was slight. For the entire group the male had a slightly greater inclination to atheroclerosis than the female. This variance was within the possibility of error, yet the individual age group differences fell beyond it.

Most authors, in comparing the incidence of atherosclerosis in males and females, do not take into consideration the severity, as the methods available make such determinations impossible. The reports are unanimous as to the predominance of atherosclerosis in the male (Jores, Cramer, E. Kaufmann). With my method both incidence and severity are considered, and although the male showed the greater tendency to atherosclerosis, it was only in the second to fourth decades.

For the colored race the female presented a more obtuse fat angle of the aorta (F.A.A.) up to 50 years, after which time the difference was negligible. When the possibility of error was considered, the only

dissimilarity was between 41 and 50 years, at which time it reached a 33 per cent variation in favor of the female (syphilis of the aorta included). The fat angles of the aorta (F.A.A.) for the male between 41 and 60 years fell below the expected values owing to the exclusion of syphilis of the aorta (cf. earlier paragraph).

TABLE 11.—*The Fat Angle of the Aorta (F.A.A.) for Smooth Aortas*

	White			Colored		
Age	Cases	Fat, Gm.	F. A. A., Degrees	Cases	Fat, Gm.	F. A. A., Degrees
25–30	15	0.065	22.2	21	0.072	24.4
31–40	32	0.080	16.8	24	0.093	21.4
41–50	36	0.137	21.2	18	0.113	17.2
51–60	21	0.145	17.4	6	0.128	15.2
61–70	3	0.182	17.9	3	0.186	18.4
71–						
	Average F. A. A.....		19.3	Average F. A. A.....		19.3

TABLE 12.—*The Fat Angle of the Aorta (F.A.A.) for Slight to Moderate Atherosclerosis*

	White			Colored		
Age	Cases	Fat, Gm.	F. A. A., Degrees	Cases	Fat, Gm.	F. A. A., Degrees
25–30				4	0.215	55.2
31–40	8	0.202	42.3	14	0.299	54.3
41–50	34	0.334	46.3	8	0.301	43
51–60	36	0.440	54.5	15	0.463	48.9
61–70	29	0.517	44.9	5	0.645	51.2
71–	16	0.606	44.5	2	0.654	46.5
	Average F. A. A.....		46.5	Average F. A. A.....		49.8

TABLE 13.—*The Fat Angle of the Aorta (F.A.A.) for Moderate to Severe Atherosclerosis*

	White			Colored		
Age	Cases	Fat, Gm.	F. A. A., Degrees	Cases	Fat, Gm.	F. A. A., Degrees
25–30				2	0.548	76.8
31–40	4	0.662	72.7	4	0.678	73.1
41–50	11	1.146	75.1	10	0.827	69.8
51–60	14	1.182	71	4	0.957	66.9
61–70	25	1.275	68.4	7	1.359	73.2
71–	22	1.496	67.9			
	Average F. A. A.....		71	Average F. A. A.....		71.9

In order to establish a correlation between the fat angle of the aorta (F.A.A.) and the intensity of the atherosclerosis, the degree of inclination for the values in part I B was determined. Because a larger number of cases were desirable for comparison, the sexes of each race were considered as one (tables 11 to 13).

The average fat angles for both races were similar. The smooth aortas had a fat angle of 19.3°, the aortas showing slight to moderate

atherosclerosis an angle of 48.1°, and the aortas showing moderate to severe atherosclerosis an angle of 71.4° (fig. 2).

From these values it was deducted that, for the average, the colored race living in Chicago begins to present a slight to moderate atherosclerosis at from 25 to 30 years, while for the white race the first indication appears between 31 and 40 years. When the races were considered as a whole, the average person over 25 years coming to autopsy at the Cook County Hospital had a slight to moderate atherosclerosis.

Comment.—From the evidence offered in part C, the results of parts A and B take shape and form, so to speak. In other words, the fat angle of any group being known, an insight into the incidence and severity of atherosclerosis in the group is obtained.

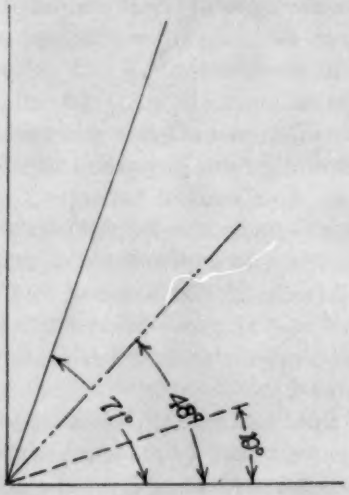

Fig. 2.—The fat angle of the aorta (F.A.A.): Angle of 71°, moderate to severe atherosclerosis; angle of 48°, slight to moderate atherosclerosis; angle of 19°, smooth aortas.

The nature of the curves and the calculation of their angles of inclination seemed to indicate that the accession of atherosclerosis is related only to age. On closer inspection, however, the differences noted with the two races and sexes indicated that other factors were at play. As has already been suggested, the cholesterol metabolism, the physical and chemical changes of the aortic wall and heredity all play their respective rôle. With the standard of measurement presented the influence of these factors can be judged more accurately. It is hoped that with this standard of measurement similar studies will be carried out by other authors, as it is only by exact methods of comparison that definite conclusions can be drawn.

Summary.—A simple chemical method has been described to determine the fat content of the aorta. The latter was found to be directly proportional to the severity of atherosclerosis in an examination of 500 aortas.

Because in a homogeneous group the increase of fat in the aorta followed an almost regular progression with age, the inclination of this ascent could be determined by the formula:

$$\text{tangent of the fat angle of the aorta (F.A.A.)} = \frac{(\text{grams of fat} \times 100) - 2}{\text{age} - 15}$$

The value thus obtained was designated as the fat angle of the aorta (F.A.A.) or the inclination to atherosclerosis. The advantage of this method is that it interprets both the incidence and the severity of atherosclerosis when a large group is considered as a whole.

It was found that an angle of 19.3° indicated a smooth aorta, an angle of 48.1° a slight to moderate atherosclerosis, and an angle of 71.4°, a moderate to severe atherosclerosis.

The fat angle of the aorta (F.A.A.) for all cases examined was 49.1° or, translated, a slight to moderate atherosclerosis.

The colored race had a greater inclination to atherosclerosis than the white race, which was most marked between 25 and 40 years. The white race developed atherosclerosis later than the colored race.

The white female had a lesser tendency to atherosclerosis than the white male but only between 25 and 40 years.

The colored race showed no great difference in their F.A.A. with sex except between 41 and 50 years, when the female showed a predominant amount of atherosclerosis.

It was suggested that with the aforesaid standard of measurement the relationship of atherosclerosis of the aorta to the cholesterol metabolism, the physical and chemical changes of the aorta and the heredity may be better understood.

II. BEARING OF CHOLESTEROL METABOLISM ON INCLINATION TO ATHEROSCLEROSIS OF THE AORTA (F.A.A.)

Knowledge of the cholesterol metabolism is limited. What determines the blood cholesterol is equally obscure. It is customary to speak of oxogenous and endogenous cholesterol.

Although it is generally accepted that the cholesterol of the blood remains constant irrespective of the diet (Bürger, Beumer, Thannhauser) the endogenous cholesterol is indirectly dependent on it. Thannhauser expressed the belief that the endogenous cholesterol in adult human beings is obtained more from the fat depots than by synthesis.

In infants Beumer demonstrated that, notwithstanding the presence of a negative cholesterol balance, the cholesterol of the brain increases twelvefold in the first eighteen months. In herbivorous animals the synthesis of cholesterol in the body has been more definitely established.

Schönheimer has shown in rabbits that the vegetable sterols (phytosterol) are not absorbable, yet cholesterol is present in the rabbit's blood.

Whatever the relationship between the endogenous and exogenous cholesterol may be, accurate studies seem to indicate that the former is to a great extent dependent on the latter, although the influence may not be a direct one (Bürger, Beumer, Thannhauser,[b] Schönheimer[a]). The term "endogenous cholesterol" as used in this paper refers to that cholesterol not derived directly from the nutrition.

A. EXOGENOUS CHOLESTEROL

If any relationship could be established between the diet, especially as it affects the cholesterol metabolism, and atherosclerosis, a great stride forward would have been made toward the solution of the pathogenesis and prevention of the disease.

From an experimental standpoint the production of atheromatous lesions in the aorta of the rabbit by feeding cholesterol dissolved in oil was first shown by Anitschkow and Chalatow and verified by numerous others (Wacker and Hueck, Bailey, Versé, Schönheimer,[a, b, c] Thölldte, Rosenthal).

The objections to comparing the experimental lesions to those found in man are that a severe hypercholesteremia is produced in the rabbit, rarely found in man, and that not only the aorta but the pulmonary artery, as well as the veins (Schönheimer[b]) and other organs, show deposition of cholesterol. The lesions in the experimental animals supposedly represent only the lipoid deposits seen in nursing infants, at puberty, etc. (Zinzerling,[a] Aschoff,[b] Klotz and Manning).

Many objections of the types stated have been overruled by further experiments. Thus Anitschkow,[c] by feeding rabbits diluted milk and egg yolk over a period of two and a half years, found a mild type of atherosclerosis with only a slight elevation of the blood cholesterol and slight to no deposits in other organs. Similar results were obtained by Chuma and Zinzerling,[b] who fed hydrous wool fat to rabbits over a long period.

The relationship of the fatty streaks and plaques in youth to atherosclerosis has also been much disputed. Virchow believed them to be separate processes (also Ribbert, Klotz and Manning, Beitzke, Lubarsch, E. Kaufmann), but the recent thorough work of Zinzerling,[a] who stained human aortas of various ages in situ with sudan III, has shown that these fatty deposits are found in the same locality as the atherosclerotic ones, and that the two processes are definitely connected (Aschoff,[c, e, f] Anitschkow[c]).

The foregoing comparison was not given with a view to showing that experimental and human atherosclerosis are similar, as there are many differences indeed. Anitschkow[c] himself showed that in experimental

atherosclerosis the elastic lamellae are never infiltrated as in man, and that the hyaline plaque and scar formation, as well as the ulceration, are rarely seen in the experimental animal. What is stressed here is that the similarities which are present are of such a character that one must admit that cholesterol (especially the esters) plays some rôle in the pathogenesis of atherosclerosis.

In dogs and other carnivora the enteral administration of cholesterol does not produce atherosclerosis, especially in young animals (Anitschkow[b] Kawamura, Cirio, Yuasa, Adler, Tsunoda and Umehara). This is because carnivorous animals excrete their cholesterol very rapidly (Rothschild) in contradistinction from herbivorous animals, which retain it. Only when the cholesterol can be fixed in the blood do atheromatous lesions occur (as after castration—Murata and Kataoka,[a] Löwenthal). This does not disprove that enteral cholesterol may produce atherosclerosis, as the experiments on dogs have been carried on for relatively only a short time (two years—Tsunoda and Umehara), and a type of atherosclerosis does occur spontaneously in old dogs (Zinzerling,[b] Krause).

In man the same disposition to excrete cholesterol exists as in other carnivorous animals. Many authors believe that enteral cholesterol does not affect the cholesterol content of the blood at all. Ssokoloff obtained no digestive hypercholesteremia in normal men after administering 3 Gm. of cholesterol dissolved in oil daily for three days. His examinations of the blood were made after twenty-four hours. Mjassnikow obtained negative results in normal men after feeding 2 Gm. of cholesterol in the form of eggs or their equivalent. He examined the blood of his patients from two to three hours after feeding, using a colorimetric method of determining the cholesterol. In a similar way, Rouzaud and Cabanis obtained no digestive lipemia. However, the methods employed by the aforementioned authors were faulty, either in the amount of cholesterol fed, the time after feeding at which determinations of the blood cholesterol were made, or the methods used for determining the cholesterol.

Bürger uses the following method of examining his patient:

1. At least 5 Gm. of cholesterol dissolved in 100 cc. of oil at body temperature must be given on a fasting stomach.

2. The determinations of the blood cholesterol should be carried out at four, eight and twenty-four hour intervals.

3. The Windaus digitonin method for determining blood cholesterol must be employed, as colorimetric methods may obscure the results, especially if the serum is highly colored.

Following this method, Bürger found that in all adults examined the blood cholesterol increased over 100 per cent in four hours; the relationship of free cholesterol to cholesterol esters remained constant. In

eight hours the blood cholesterol was 50 per cent above normal, and in twenty-four hours it was normal. Positive results were obtained by Widal, Weil and Laudot and Arndt.

Barreda from Thannhauser's clinic recently repeated Bürger's work, but no determinations were made after eight hours. In normal persons he found an increase of free cholesterol of from 7 to 25 per cent (in 5 of 6 cases) and an increase of total cholesterol of from 6 to 17 per cent (in 4 of 6 cases). These readings were after four hours.

It follows then that there is a distinct digestive lipemia in man, and that its peak is in four hours. Considering that as a rule one eats every four to five hours, it may be inferred that, depending on the type of food eaten, an almost constant lipemia may be present during the day.

A large part of the exogenous cholesterol passes through the body of the carnivorous animal, although it is rapidly excreted. The purpose of the cycle of intestine to thoracic duct, to blood, to liver, to bile, to intestine is not understood. As will be shown later, atherosclerosis of the aorta as it occurs in man is never found in animals which are fed a diet devoid of cholesterol and which must synthesize their own sterols. Can one assume that exogenous cholesterol, although not to be differentiated from the endogenous type, may nevertheless have singular properties not yet recognizable?

If, on the basis of the foregoing hypothesis, the atherosclerosis may be influenced by the type of cholesterol, and the latter in turn is partly determined by the diet, an avenue is opened for a research that should be encouraged.

The first large stride in that direction has been taken by Raab [b] who, by personal information and through the literature, has gathered all available data concerning the interconnections of diet and atherosclerosis. Table 14 is a compilation of his results as well as of additional information obtained in the present investigation.

On scanning this table one notices that the accounts are based, to a great extent, on clinical observations, and that emphasis is placed on protein diet and elevation of blood pressure. This may be explained by the evidence of the earlier workers on experimental atherosclerosis who utilized animal tissues (Ignatowsky, Steinbiss and Stuckey). It was then thought that the high protein content was the active principle, but it was later proved that the cholesterol component was the important one (Stuckey, Anitschkow,[a] Chalatow,[a] Wacker and Hueck). Similarly, the blood pressure was thought to be influenced by a high protein diet. There have been many studies made disproving this conception, the most striking being that of Thomas. He found that Eskimos, who live mainly on red meat, have an average blood pressure between 40 and 60 years of 129 systolic and 76 diastolic. This pressure is much lower than that found in the United States (Gager, Foster).

TABLE 14.—*The Relationship of Diet and Blood Pressure to Atherosclerosis*

	Race	Diet: Protein	Diet: Fat	Diet: Carbohydrate	Blood Pressure, Mm. of Mercury	Incidence of Atherosclerosis	Comment	Authors and References
1	White, Europe	Low, no meat or eggs	Low, no butter, little milk	Moderate	75% under 120	Not given	Monks who rarely talk	Saile
2	White, Europe	High, meat and eggs	High, butter and milk	High	70% over 120 between 50 and 90 yrs., 140–160	Not given	Monks who go among their people	Saile
3	White, Germany	Low, very little fat of any kind	Low	Moderate	.....	Much decreased	During and shortly after the war	Aschoff (*f*)
4	White, Vienna, Austria	Normal European diet			.....	40% of autopsies in older persons, up to 88%	1921-1923	Schubert, F.: Klin. Wchnschr. **31**: 751, 1925
5	White, England	Normal European diet			140/90 at 60 yrs.	High	Clinical and post mortem	Donnison Roger
6	White, S. America	Very high, meat 30% of diet, also eggs 85 Gm. per day	High	High	Very high (?)	High (?)	Clinical and post mortem	Castex, S.: La hypertension arterial, Buenos Aires, H. Andretta, 1929
7	White, New York	High	High	High	23% over 150 at 40 to 60 yrs.	High	Private (clinical) practice	Gayer
8	White, U. S. A.	High	High	High	High in 5 million out of 120 million	High	Clinical	Mayors, quoted by Castex
9	White, U. S. A.	High	High	High	High	40 yrs., 11.4% 50 yrs., 26.3% 60 yrs., 39%	From death certificates (mostly clinical)	Stocks
10	White, U. S. A.	Average American diet			Average American	20-29 yrs., 3.½% 30-39 yrs., 9.¼% 40-49 yrs., 26% 50-59 yrs., 48% 60-69 yrs., 78% 70 yrs. and over, 90%	Postmortem records	Ophüls
11	White, U. S. A.	Average American diet			45% over 130 22% over 140	Negligible	Freshman class Univ. of Calif.	Alvarez, Wiezen and Mahoney
12	White, U. S. A.	Normal American diet			16% over 140 (1923) 90% over 140 (1924)	Negligible	Freshman class, Univ. of Michigan	Diehl and Sutherland
13	White, U. S. A.	Normal American diet			10% over 140	Negligible	Freshman, Harvard University	Palmer
14	White, Eskimos	High, red meat and liver	Low	Low	40 to 60 yrs., 129/76	Negligible	Clinical diagnosis, diet optimum	Thomas
15	White, Egypt	High	High	High	35 to 55 yrs., 10% hypertension	Frequent	Private practice, clinical	Ismail
16	White, Egypt	Low	Low	High	No hypertension	No atherosclerosis	Hospital practice, poor class	Ismail

TABLE 14.—*The Relationship of Diet and Blood Pressure to Atherosclerosis—Continued*

	Race	Diet: Protein	Diet: Fat	Diet: Carbohydrate	Blood Pressure, Mm. of Mercury	Incidence of Atherosclerosis	Comment	Authors and References
17	Brown, Indians, Calcutta	Low	Low	High	Lower than Europeans	Similar to Europeans	Postmortem material	Roger
18	Brown, Indians, British	7.0%	1.4%	91%	Low	50% lower than in Europe	Eggs, milk, butter in background; i.e., little of eggs, milk and butter; many cereals (poor class)	McCarrison and Rai, quoted by Raab (*b*)
								Raab (*b*)
19	Brown, Indians, British	9.4%	10.6%	80%	High	.....	Well to do class	Raab (*b*)
20	Brown, Malay, Dutch East Indies	Low	Low	High	Low	Very seldom	Blood cholesterol as well as calcium low	de Langen
21	Yellow, Kirgisen-Steppe	Very high, 10-20 lbs. of goat meat	High (mare milk)	High	.....	High	Apoplexy and contracted kidneys common	Kucsynski
22	Yellow, Chinese	9%	4% (plant)	87%	5 to 10 lower than white	Infrequent	Clinical	Tung (*b*) Maxwell, quoted by Raab (*b*)
23	Yellow, Chinese	Low	Low	High	Among 4,000 patients 20 over 160	Infrequent	Clinical .	Foster
24	Yellow, Chinese	Low	Low	High	Low	Among 1,924 patients 50 with atherosclerosis	Clinical	Personal communication of Dienarde with Raab
25	Yellow, Japanese	13.7%	4.9%	81.4%	Relatively low	Diseases of arteries, 21.8%	Statistics from insurance company	Rubner, quoted by Raab (*b*)
26	Black, East Africa	Low	Low	High	60 yrs., 105/67	Negligible	Clinical and post mortem	Donnison
27	Black, Jamaica	Moderate	Moderate	High	33% over 140	Frequent	.....	E. T. Newmann
28	Black, U. S. A.	.....	.....	.....	.....	40 yrs., 14.8% 50 yrs., 31.0% 60 yrs., 45.0%	Death certificate diagnosis	Stocks

Increased blood pressure definitely predisposes to atherosclerosis, but one cannot imply that the two conditions are concomitant. (See Alvarez, Wiezen and Mahoney, Diehl and Sutherland, and Palmer in table 14 for reports on freshman university students.) Thus, in spite of the large material gathered, accurate deductions cannot be made. Yet there are many observations which suggest that precise information would be of great value, and that further work along similar lines but with a definite standard of measurement should be encouraged.

One of the outstanding features of table 14 is that in no race for which a high cholesterol intake (in the form of eggs, butter and milk)

and fat intake are recorded is atherosclerosis absent (America, United Kingdom, Central Europe, South America, Mongolia: Majors, Rogers, Schubert, Castex, Kuczynski, respectively). Where a high protein diet is consumed, which naturally contains small quantities of cholesterol, but where the neutral fat intake is low, atherosclerosis is not prevalent. Thus, in the Japanese race, by which a diet rich in protein but low in neutral fat (4.9 per cent compared with 11.4 per cent in the western diets—Rubner) is consumed, the incidence of atherosclerosis is much lower than it is in the United States, e. g. (3.1 to 21 per cent, respectively—Rubner). Similarly, the Eskimos who, contrary to current opinion, eat very little neutral fat (as the oils extracted from the liver, etc., are used for heating, lighting and cooking—Thomas) have a low incidence of atherosclerosis, although their diet consists mainly of red meat (Thomas).

As mentioned by Hoppe-Seyler in 1857 and verified by Versé,[8] Schönheimer,[8] Thannhauser, Bürger and others, the neutral fat paves the way for cholesterol absorption. Diets high in cholesterol and low in neutral fat may result in a much lower blood cholesterol than a diet high in neutral fat and low in cholesterol. This has been proved definitely experimentally (Versé, Wacker and Hueck). Aschoff[1] traces the marked decrease of atherosclerosis in Central Europe following the war to the low intake of fat.

Of interest is the report of Ismail in Egypt, who has communicated that among his private patients, whose diet is similar to that of the Europeans, the incidence of atherosclerosis is high, while in his hospital practice composed mainly of natives, who subsist largely on a carbohydrate diet, the incidence of atherosclerosis is low. Here one has people living under similar conditions of climate and environment, but consuming different foods, with a marked variation in their predisposition to atherosclerosis.

Saile compared monks living on an absolute vegetarian diet (without meat, eggs or butter) with another group of monks who were nonvegetarian, subsisting on a diet similar to that of the average European. Unfortunately, his results dealt mainly with blood pressure; he showed that only 25.4 per cent of the vegetarians had a systolic blood pressure over 120 mm. of mercury. In comparison, 70.4 per cent of the nonvegetarians had a blood pressure over that amount. He inferred a similar relationship to arterial changes. It must be stressed at this point that the vegetarians usually considered in the literature abstain from meat but consume eggs, milk and butter to a high degree. This diet, high in cholesterol and neutral fat, may account for the contradictory opinions.

One discrepancy was reported by Roger, who found that the incidence of atherosclerosis in Bengal Indians was similar to that in England. The Indians eat a diet comparatively low in protein and fat, very similar to that of the southern Chinese, in whom the incidence of atherosclerosis is low (Foster). It is especially in such cases that an accurate method of measurement of atherosclerosis is needed.

In animals the only lesions that resemble human atherosclerosis of the aorta were found in aves, especially parrots (Fox, Wolkoff,[8] Nieberle and Beneke). These birds are meat and seed eaters, and thus cholesterol and neutral fats, respectively, are plentiful in their diet. That other animals also consume meat and fat (gormandizing animals) and rarely develop atherosclerosis is true, as it cannot be denied that age and construction of the aorta (Fox) play a definite rôle, yet it is significant that those who do acquire an atherosclerosis similar to that in man consume cholesterol and neutral fats.

As has been stated, the foregoing statistics cannot be considered as final, as no definite or accurate standard of measurement was employed, but they offer some hope that cholesterol and neutral fat in the diet may influence the inclination to atherosclerosis, and that further work should be carried on in which a common exact method of comparison is used. The method suggested in part I for the determination of the inclination to atherosclerosis is offered as a means to this end.

B. A COMPARISON OF THE DIETS OF THE COLORED AND THE WHITE RACE AND ITS BEARING ON THE FAT ANGLE OF THE AORTA (F.A.A.)

The fat angles of the aorta (F.A.A.) for both races and sexes were equal, except for the discrepancy between 25 and 40 years. In seeking for the cause of this difference, several possibilities presented themselves.

The persons of the colored race of Chicago considered in this paper were for the greater part southern-born, and in determining the number of years that each group had resided in Chicago it was found that this was roughly proportional to their age (table 15).

TABLE 15.—*The Average Number of Years of Residence in Chicago of the Colored Race*

Age	Male	Female
25–30	6 yrs.	12 yrs.
31–40	13 yrs.	12 yrs.
41–50	16 yrs.	10 yrs.
51–60	22 yrs.	9 yrs.
61–70	22 yrs.	27 yrs.
71–	25 yrs.	40 yrs.

Thus, up to 40 years for the male and up to 60 years for the female the average residence in Chicago was relatively short. Considering that the colored people of the South are very poor and live in a rather primitive manner, deficiency diseases are exceedingly common there.

In an effort to ascertain the nature of the diet of the southern colored people, a questionnaire was sent out to various Negro institutions of the South. Answers were received from Louisiana, Alabama, North Carolina and Texas.

The general opinion of these reports from the Negroes was that the greatest proportion of their diets (as with all poor people) consisted of carbohydrates (cornbread, potatoes, molasses). To a much less extent meat was consumed, and butter, milk and eggs were eaten in minimal quantities.

When the colored persons came to the North their standard of living was immediately elevated. Although a large proportion of them remained very poor, their labors brought them higher returns, and what they themselves could not supply, society supplied for them. Many of the colored persons entering the Cook County Hospital are comparatively well-to-do, however, and their consumption of meat, milk, eggs and butter approaches and to some extent surpasses that of the white persons interned at the hospital. Although the diet of the foreign white persons had also changed, the difference was much less marked, as these came from Central Europe. Further, the younger white persons (from 25 to 35 years of age) are for the most part American-born.

Can this change of diet and also environment of the colored race play some rôle in their cholesterol metabolism? Whether the increase of cholesterol, neutral fats and proteins in their diets increased directly the cholesterol content of the blood, or whether this change of diet caused a disturbance of the cholesterol metabolism in general cannot be stated (Weiss and Minot). The fact remains that there is an increase of fat deposit in their aortas, and although there are no studies determining the cholesterol content of their blood, the following observation suggests that there might be an elevation.

Joël has shown by simultaneous determinations of the blood cholesterol and examinations of the cornea in young persons (about 25 years of age) that a close relationship exists. Thus he found elevations of cholesterol up to 320 mg. per hundred cubic centimeters of blood in young persons with arcus lipoides. Experimentally arcus lipoides is easily produced in rabbits by feeding them cholesterol (Versé, Kolen, Schönheimer,[8] Rosenthal). This condition in rabbits resembles almost exactly that in young persons.

A comparison of the occurrence of arcus lipoides in persons between 25 and 40 years of the two races revealed that it was not recorded as occurring in any of the white persons, whereas among the colored per-

sons it occurred in 46 per cent of the males and in 36.3 per cent of the females. In only one instance did arcus lipoides occur in a 30 year old colored man who presented an aorta with a few fatty plaques and streaks and a fat content of 0.041 Gm.

Can it be deducted on the basis of Joël's work that the cholesterol content of the blood of the colored race between 25 and 40 years is higher than that of the white race (or at least between 25 and 30 years, for on the basis of the experimental work of Kolen arcus lipoides may remain for years after the disappearance of the hypercholesteremia)? If so, perhaps the blood cholesterol does play some active rôle in atherosclerosis. Further, can one suggest that a hypercholesteremia occurs before atherosclerosis as in the case of the 30 year old colored man who had an arcus lipoides and only a few yellow streaks as well as a low fat content in his aorta?

If the blood cholesterol of the colored race is higher than that of the white race between 25 and 30 years, what can account for this difference, since the diets of the two races while living in Chicago are about similar?

Is it possible that the Negro living under rather primitive conditions in the South, where proteins and cholesterol-containing foods are luxuries, is slow, when transferred to a different environment with a higher standard of living, to accommodate himself to the new diet, and a hypercholesteremia occurs? After a longer residence acclimatization is established, and the cholesterol in the blood returns to the level of the white man.

One is tempted to compare what has been described with what occurs in the cholesterol-fed rabbit, which at first reacts with hypercholesteremia, but in which, after protracted cholesterol feeding, the cholesterol content of the blood falls to normal (Thölldte, Schönheimer,[a] Rohrschneider).

There is a strong possibility that a disturbance of the cholesterol metabolism of the Negro may have taken place before his emigration to the North. Unfortunately the only evidence that I could find regarding the incidence of atherosclerosis of the colored race in America was based on clinical diagnosis (Stocks), and the statistics showed a preponderance of atherosclerosis in the colored race. Most probably syphilitic aortitis was included in this study, and thus these values cannot be accepted. The anatomic statistics of the earlier authors (Camac) dealt mainly with syphilis.

Bearing in mind that the American colored person took his origin from North Africa not very long ago, one may ask whether this more drastic change of environment may not still be leaving its stigmas on the present colored population of America.

Reports from Africa (East African Negroes) by Donnison show that the East African, living primitively and excluding meat and cholesterol-containing food from his diet almost completely, has an almost negligible incidence of atherosclerosis.

The foregoing evidence is indicative only of the possibility that cholesterol metabolism plays a rôle in atherosclerosis of the aorta and needs further verification. The fact that increased blood pressure, to which the young Negro is susceptible, may also influence the lipoid deposit in the aorta will be discussed later.

C. THE RELATIONSHIP BETWEEN ENDOGENOUS CHOLESTEROL AND THE FAT ANGLE OF THE AORTA (F.A.A.)

Experimental and clinical observations strongly suggest that the body synthesizes a portion of its cholesterol (Beumer and Schönheimer [e]). In human adults this fraction is very small or may even be transported from the fat depots (Thannhauser). In spite of the varying amounts of cholesterol obtained from the diet (0.018 to 1.4 Gm., depending on the cholesterol content of the diet—Thannhauser) and the endogenous quota (0.03 Gm.—Thannhauser) the blood cholesterol remains constant. The regulating mechanism is believed to be in the liver (Bürger, Thannhauser [b]).

Hypercholesteremia from an endogenous origin may be divided into the following groups (Bürger):

1. Hypercholesteremia of pregnancy
2. Transport hypercholesteremia
3. Retention hypercholesteremia
4. Cytolytic hypercholesteremia
5. Narcosis hypercholesteremia

As hypercholesteremia, in order to effect a lipoid deposit in the aorta, must act over a longer period of time, the interest here lies in the second, third and fourth divisions.

Transport Hypercholesteremia.—The hypercholesteremia of this group is supposedly due to the lipemia from inanition or hunger (Schultze, Thannhauser,[b] Bürger). The theory is that with inanition the fat is transported from the fat depots by way of the blood stream to the parenchyma of the body. In the course of this, the cholesterol present in the fat depots is also carried into the blood. It does not follow that with a given amount of neutral fat there will be a corresponding amount of cholesterol transported. In tuberculosis and carcinoma, e. g., the cholesterol of the mesenteric and subcutaneous fat becomes more concentrated (Wacker) with inanition. If this inanition or hunger is protracted over a longer period, an exhaustion of the lipoid

and cholesterol depots takes place and a hypocholesteremia may set in, as found in hunger edema (Knack and Neumann; Feigl and Mathies, cited by Bürger).

Under transport hypercholesteremia Thannhauser included atherosclerosis, diabetes mellitus, chronic glomerular nephritis, tuberculosis and carcinoma. Although there are other factors at work influencing this hypercholesteremia, such as acidosis in diabetes mellitus, their exact nature is not known.

(*a*) Atherosclerosis of the Aorta: Hypercholesteremia in atherosclerosis has been reported (Mjassnikow, Pribram) as well as normal or lower than normal blood cholesterol values (Hunt). The negative reports do not exclude the possibility that a hypercholesteremia might have existed for some time previously, and that the damage had already been done. The deposition of cholesterol and cholesterol esters, once it has occurred in the aorta of the adult person, is not reversible (Aschoff [4]).

Another possibility is that lipemia aids in producing a steatosis, as has been shown experimentally (Versé [3]). A relatively low cholesterol content may be more effective if a lipemia is associated.

The likelihood that in the young colored persons a hypercholesteremia may have accounted for the greater inclination to atherosclerosis has already been discussed.

In the following diseases the fat angles are given for the entire group studied because of the lack of material. The average values given were so calculated as to correspond with the number of cases compared.

(*b*) Diabetes Mellitus: In diabetes mellitus the blood cholesterol and also the neutral fats are usually high (Klemper and Umbar, quoted by Bürger), especially when atherosclerosis of the aorta is associated (Joslin, Weiss and Minot). Klemper and Umbar, as well as Bürger, found that an acidosis was usually associated in cases of lipocholesteremia, although not always. Normal values of blood lipoid and cholesterol may be found in diabetes, especially when acidosis is not present. The low cholesterol values obtained by Hunt in diabetes mellitus with atherosclerosis of the aorta may be explained by the absence of acidosis at the time of examination, but they do not exclude the possibility that a hyperlipocholesteremia existed at some previous time when acidosis was present.

The finding of a hyperlipocholesteremia does not necessarily mean that an atherosclerosis of the aorta will follow, unless it is protracted over a long period. Joslin reports an increase of atherosclerosis in diabetes mellitus of from 30 to 68 per cent because the length of life has been increased from five to thirty years owing to the event of insulin. Whether it is the hypercholesteremia or whether it is an alteration of the physical and chemical properties of the cholesterol ester resulting

from the disturbance of the cholesteral metabolism that influences the development of atherosclerosis of the aorta is not known.

The 8 persons examined for fat in the aorta were grouped together because of the lack of material (6 white and 2 colored persons).

Between 31 and 40 years there was 1 person with a fat angle of 53.4° as compared with 44.3° of the composite group (table 16). Between 41 and 50 years the F.A.A. was 66.9° as compared with 48.2°, and between 51 and 65 years it was 59° as compared with 54°. The average F.A.A. for diabetes mellitus was 59.7°, while for the composite group it was 48.8°.

In other words, the inclination to atherosclerosis of the aorta was much greater in those with diabetes mellitus than in the average group.

TABLE 16.—*The F.A.A. in Diabetes Mellitus*

Age	Cases	Fat, Gm.	F. A. A., Degrees	Average F. A. A.
31–40	1	0.288	53.4	44.3
41–50	3	0.720	66.9	48.2
51–65	4	0.751	59.0	54.0
		Average	59.7	48.8

TABLE 17.—*The F.A.A. in Chronic Glomerular Nephritis*

Age	Cases	Fat, Gm.	F. A. A., Degrees	Average F. A. A.
25–30	2	0.127	40.6	29.5
31–40	2	0.317	56.0	47.4
41–50	5	0.540	60.0	51.0
51–60	3	0.701	59.8	49.6
61–70				
71–	1	1.366	63.3	60.5
		Average	55.9	47.6

As the F.A.A. was 59.7°, this would indicate that the average diabetic patient examined at the Cook County Hospital has a moderate atherosclerosis. Unfortunately, the small number of cases makes the possibility of error very great. Yet the values given are far beyond the zone of error.

(*c*) Chronic Glomerular Nephritis: Bürger found high normal values of blood cholesterol in chronic glomerular nephritis (200 mg. per hundred cubic centimeters of blood) using the Windaus digitonin method for the determination of these values. Other authors using a similar method have reported high normal or definitely increased values.

Thirteen persons with chronic glomerular nephritis were examined (5 white and 8 colored persons). The F.A.A.s are given in table 17. In comparing these results with those for the composite group it was noted that for every age the fat angle was higher in nephritis. The

average F.A.A. for nephritis was 55.9°, while for the composite group it was 47.6°, a 20 per cent difference.

Considering that the blood cholesterol was unchanged or only slightly elevated, the 20 per cent above the average inclination indicated that some other factor must have been present to account for this difference. The latter factor lies in the blood pressure and will be discussed in a later chapter.

(*d*) Chronic Tuberculosis: In chronic tuberculosis the cholesterol metabolism may be affected in many ways. When inanition sets in, a hypercholesteremia may be present, which may then be accelerated by a cytolytic factor due to destruction of tissue. With extreme inanition the exhaustion of fat as well as of cholesterol may lead to a hypocholesteremia, especially when a severe anemia is associated (Rosenthal and Patrzek).

The 25 persons with chronic tuberculosis (in 19 of whom the condition was pulmonary) were grouped together (8 white and 17 colored).

TABLE 18.—*The F.A.A. in Chronic Tuberculosis*

Age	Cases	Fat, Gm.	F. A. A., Degrees	Average F. A. A.	Weight, Lbs.
25–30	8	0.063	19.2	33.0	94
31–40	5	0.167	36.4	41.0	113
41–50	2	0.276	40.5	49.0	121
51–60	5	0.550	53.0	49.0	91
61–70	5	0.807	57.7	58.4	96
		Average....	41.3	46.1	

Except for the first age group (from 25 to 30 years), the average F.A.A. did not vary greatly (table 18). Although inanition was present in most of the cases, it was not so marked that a hunger edema had set in. The average body weights given in table 5 demonstrate this point. Thus one might infer that there was some disturbance in the cholesterol metabolism.

The average inclination to atherosclerosis in persons with chronic tuberculosis was slightly lower (41.3°) than that of the average group (46.1°). A slight disturbance of the cholesterol metabolism, then, does not lead to atherosclerosis of the aorta, other factors being necessary.

(*e*) Carcinoma: In carcinoma, as in tuberculosis, the hypercholesteremia is supposedly due to the lipemia from inanition and cellular destruction. Because carcinoma cells contain a high percentage of cholesterol (Wilheim and Fuchs), necrosis of the latter with absorption should lead to an even higher cholesterol content of the blood than in tuberculosis.

Among the 81 persons examined there were 61 white and 14 colored persons. Naturally the results for the white will be more accurate than those for the colored persons.

A slight difference was noted in the fat angles of the aorta (F.A.A.) for the various groups of white persons and for the entire group as compared with the average. The inclination to atherosclerosis was lower for the carcinoma group (table 19).

For the colored race the F.A.A. was much lower after the age of 30 than the average. Considering that a disturbance of the cholesterol metabolism was present, one would expect a greater inclination to atherosclerosis of the aorta. Here, as with tuberculosis, the blood pressure played a rôle, and it is evident that a slight disturbance of the cholesterol metabolism alone will not lead to a higher incidence of atherosclerosis of the aorta.

3. *Retention Hypercholesteremia.*—Every condition that prevents the bile from entering the intestine leads to a retention hypercholesteremia. In retention of long standing due to obstruction of the bile duct the blood cholesterol returns to normal (Stepp, Rosenthal and Holzer). The

TABLE 19.—*The F.A.A. in Carcinoma*

	White					Colored				
Age	Cases	Fat, Gm.	F. A. A., Degrees	Average F. A. A.	Weight, Lbs.	Cases	Fat, Gm.	F. A. A., Degrees	Average F. A. A.	Weight, Lbs.
21–30						3	0.167	49.5	40.7	88
31–40	7	0.141	31.0	34.0	126	3	0.081	17.0	45.0	100
41–50	17	0.259	38.5	48.4	110	5	0.273	40.2	44.0	101
51–60	22	0.324	37.3	51.0	134	3	0.263	31.3	49.7	125
61–70	14	0.804	57.5	58.4	97					
71–	7	0.768	51.3	61.4	115					
		Average	43.1	50.1			Average	34.5	45.1	

hypercholesteremia thus obtained extends over a short period of time and does not come under consideration in this paper.

(*a*) Atrophic Cirrhosis of the Liver: In atrophic cirrhosis of the liver without icterus an elevation of the blood cholesterol can also take place. The decreased amount of bile in the intestine plus the decrease in pancreatic lipase (associated cirrhosis of the pancreas) leads to a decreased absorption of cholesterol and fat, as has been shown by fat meal tests (Bürger and Habs). The blood cholesterol in atrophic cirrhosis of the liver may at first be elevated but returns to normal.

Of 7 cases of atrophic cirrhosis of the liver studied by Bürger and Habs, only 1 showed a blood cholesterol above normal, 299 mg. per hundred cubic centimeters, while the lowest value was 76 mg.

The 10 cases of atrophic cirrhosis of the liver without icterus that were studied (table 20) showed an inclination to atherosclerosis lower than the average, except between 51 and 60 years. The inclination for those with cirrhosis of the liver was about 22 per cent lower. Again the limited number of cases prevents definite conclusions, but there is a suggestion that when the cholesterol metabolism is slightly disturbed or not at all the inclination to atherosclerosis (F.A.A.) is not increased.

In atrophic cirrhosis of the liver with icterus there is a relative or an absolute decrease of the cholesterol esters in the blood (for the literature, Bürger and Thannhauser may be referred to). No such cases were available for study.

4. *Cytolytic Hypercholesteremia.*—This type of hypercholesteremia is supposedly dependent on cellular destruction. The absorption of the cholesterol-containing detritus should account for an increase of cholesterol in the blood.

In this group acute infectious diseases and lipoid nephrosis are considered. Tuberculosis and carcinoma, in which the hypercholesteremia is of mixed types, have already been discussed. Some authors consider the hypercholesteremia in the first two conditions mentioned as also of mixed types, i. e., transport and cytolytic (Bürger and Thannhauser [b]).

(*a*) Acute Infectious Diseases: Whatever the cause of the hypercholesteremia may be, it is known that during the stage of fever the blood cholesterol is low, and that after the fever subsides the blood

TABLE 20.—*The F.A.A. in Atrophic Cirrhosis of the Liver Without Icterus*

Age	Cases	Fat, Gm.	F. A. A., Degrees	Average F. A. A.
29–40	2	0.079	17.5	35.0
41–50	2	0.213	32.8	48.5
51–61	6	0.571	53.2	50.5
		Average	34.8	44.4

cholesterol rises. The more severe the infection the higher is the subsequent rise in the blood cholesterol (Grigaut and Bürger). This rise, according to the French school, is supposedly bound up with the immunologic reaction (Grigaut). Bürger and Thannhauser expressed the belief that a disturbance of the nutrition is the underlying factor.

As a rule the hypercholesteremia is of short duration and should not affect appreciably the status of the aorta. In typhoid fever, however, in which it may extend over a period of a few months, fatty deposits in the aorta are common (Jores, Ophüls). In the 88 cases of acute infectious diseases examined there were no appreciable differences in the inclination to atherosclerosis (F.A.A.) as compared with the average for the white race (table 21). In the colored race there was a somewhat lower inclination between 25 and 30 years and 41 and 50 years. This difference, as will be explained later, was due to another factor present in the average group to a greater extent than in the group under consideration, viz., increased blood pressure.

From an experimental point of view the production of atherosclerosis by injections of killed bacteria has been reported by Saltykow and Klotz. A verification of this result could not be established by Starkodamski and Ssolowjew, who injected killed staphylococci and

added Klotz' technic of suspending the animal by its hindlegs daily. Inflammatory lesions were produced in the aorta after injections of bacteria by Thérèse and by Boinet and Romary. Benson, Smith and Semenov produced atherosclerosis of the coronary arteries of the heart in rabbits by injecting killed cultures of Streptococcus viridans only when cholesterol was added to the diet.

From an anatomic standpoint MacCallum found that persons dying of infectious diseases showed no special tendency to atherosclerosis.

The studies mentioned indicate that acute infectious diseases by themselves had no definite influence on atherosclerosis of the aorta, and when all other conditions were equal, the inclination to atherosclerosis was similar to that of the average group.

(*b*) Lipoid Nephrosis: Only 1 case of lipoid nephrosis was present in this series. It occurred in a colored man 55 years of age. The fat

TABLE 21.—*The F.A.A. in Acute Infections*

	White				Colored			
Age	Cases	Fat, Gm.	F. A. A., Degrees	Average F. A. A.	Cases	Fat, Gm.	F. A. A., Degrees	Average F. A. A.
25–30	4	0.047	12.0	19.6	7	0.076	24.3	43.0
31–40	16	0.185	39.6	34.0	4	0.229	46.2	44.2
41–50	13	0.308	46.3	48.4	10	0.182	28.4	46.0
51–60	8	0.422	45.2	51.2	8	0.373	41.4	48.2
61–70	9	0.553	46.8	54.4	3	0.786	56.9	62.0
71–	6	0.961	57.8	61.4				
		Average..	41.3	45.6		Average..	39.4	49.7

content of his aorta was 0.7784 Gm., which was interpreted as a severe atherosclerosis (ulcerations were also present).

The most important argument against the cholesterol metabolism theory in atherosclerosis of the aorta is that in the cases of hypercholesteremia, notwithstanding the elevation of cholesterol in the blood from five to ten times above normal, atherosclerosis is not a common occurrence.

Several questions will have to be answered before this objection can be considered as valid.

According to Lawinowcz (quoted by Bürger), the diet does not affect the blood cholesterol in these cases. Bürger explained the hypercholesteremia on the basis of a cellular destruction resulting from the activity of some infectious or toxic agent. It is to be seen whether or not the physical colloidal properties of the cholesterol derived from cellular destruction or other endogenous means are different from those of the exogenous cholesterol. Chalatow,[a] for example, has shown that in chemically similar cholesterol esters differences in their physical colloidal state may exist under similar temperatures.

The time element in many of these cases is too short, as the patients succumb early to the disease. If their lives could be prolonged, as in diabetes mellitus, e. g., it would then be seen whether the inclination to atherosclerosis also increases as has occurred in diabetes mellitus. Of course, the added factor of age would then be present, and it cannot be denied that senescence is of the utmost significance.

Summary.—A disturbed cholesterol metabolism as designated by hypercholesteremia acting over a long period of time tends to increase the inclination to atherosclerosis of the aorta (diabetes mellitus, lipoid nephrosis). An increased inclination to atherosclerosis of the aorta may be present without a marked hypercholesteremia (chronic glomerular nephritis). A slight increase in the blood cholesterol may not necessarily lead to an increased inclination to atherosclerosis, but possibly to a decreased inclination if the other influencing factors are wanting (tuberculosis, carcinoma, acute infectious disease).

Comment.—With a standard method of determining the inclination to atherosclerosis of the aorta (F.A.A.), a comparable method is needed for determining the cholesterol of the blood. Because of the various procedures in use for estimation of the blood cholesterol, the "normal" value shows variations from 85 to 310 mg. per hundred cubic centimeters of blood (Bang, Bloor methods). Such a marked variation speaks for a lack of uniformity either in the method employed or in the preparation of the patient before blood is taken for examination.

The recommendations given by Bürger for the preparation of the patient are given in detail because they are the best grounded.

> The patient should not have eaten more than the average amount of fat and cholesterol (i. e., not over 100 Gm. of butter or over 3 eggs or their equivalent) the day before examination, and the last intake of fat should have been at least twelve hours before the blood is drawn. The serum thus obtained should be clear and transparent. The digitonin method as described by Windaus should be employed in the determination of the cholesterol.

There are no reports covering a large series of cases in which the aforementioned method was employed. That great fluctuations of the blood cholesterol according to age occur has been shown by György, who found in examining mother and child that the blood cholesterol of the child was about one-third that of its mother. An increase of the blood cholesterol after the first year has been demonstrated by Knauer. In adult life the variations are not so striking, but if differences occur, they are of great significance. Negative results would not, however, disprove the rôle of cholesterol in the production of atherosclerosis of the aorta, as it is possible that a disturbance of the cholesterol metabolism without an actual increase in the blood cholesterol may so alter the physical colloidal properties of the cholesterol (Chalatow [8]) that the end-result

would be similar or even greater. As will be discussed later, other factors enter in with age, such as the binding or precipitation of cholesterol in the aortic wall.

Only with such standard methods as those described can advances be made in the study of the rôle of the cholesterol metabolism in atherosclerosis.

In determining what part the diet plays in atherosclerosis it must be borne in mind that important factors are the amounts of cholesterol and of neutral fats consumed. Tables giving the percentage of cholesterol in various foods may be had in the work of McCollum and Simmonds, Thannhauser, and Bürger. The stress that the earlier authors placed on the protein content of the diet is not warranted, although proteins may elevate the blood cholesterol to some extent (Newburgh and Clarkson).

Subjects for study could be had in the various races who live on different types of food. More extensive studies could more readily be carried out with different religious sects who practice dietary restrictions. The monks studied by Saile are a good sample.

One attempt has been made to determine the blood cholesterol in natives of the Netherland Indies, whose diet is composed mainly of rice. De Langen found the blood cholesterol as well as the incidence of atherosclerosis to be low. Although this work was not carried out according to the standards indicated, it suggests that work in that direction may be fruitful.

Studies concerning endogenous cholesterol and atherosclerosis of the aorta can be more readily executed in large hospitals.

The plotting of the fat angles of the aorta as well as of the cholesterol angles of the blood for a large series of cases of each of various diseases would be exceedingly instructive. The values given in the text can be taken only as an indication of the possibilities, as the cases of many of the diseases examined were too few, and thus the results were subject to much error.

In conclusion, it appears that a disturbance of the cholesterol metabolism as determined by hypercholesteremia, whether of endogenous or exogenous source, may alter the inclination to atherosclerosis.

(To be continued)

SPECIFIC CHEMOTHERAPY FOR CANCER

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If malignancy is to be regarded as the result of fundamental metabolic abnormality through which cancer cells achieve their peculiar alterations of behavior, as seems probable, there is a possibility that the abnormality may be used as a basis for specific chemotherapeutic measures. Several of these have been suggested. Fischer-Wasels,[1] who was among the first to recognize this possibility, believed that it might be feasible to effect the cure of cancer by means of the intensive administration of oxygen. More recently, Roffo [2] attempted interference with the oxidoreductive mechanisms within the cancer cell, and Goldfeder [3] reported attempts at affecting the cancer cell by alteration of its hydrogen ion content.

A simpler line of attack would appear to be possible through the peculiarity of carbohydrate metabolism discovered by Warburg.[4] Either through the altered mode of utilization of dextrose in cancer tissue or through the altered affinity of such tissue for carbohydrate, it is conceivably possible to effect its selective intoxication by means of sugar compounds as carriers of toxic radicals. That some cancers at least show an increased affinity for carbohydrate was demonstrated in 1923 by Braunstein,[5] who observed that with the onset of cancer diabetic glycosuria frequently disappears. However, some results obtained in the course of the work reported here would apparently suggest that this relation is a variable one.

Theoretically, an ideal agent of this sort would be one in which the toxic radical is linked to one of the side-chains of the dextrose molecule. At present there does not appear to be any method by which a compound of this character can be prepared, and one is practically restricted to compounds in which acids derived from sugar are combined with a basic intoxicant radical. Also, the choice of the latter is somewhat limited, and in this work two only were used—lead and arsenic in basic form (tetramethylarsonium). While any of the arsonium compounds

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1. Fischer-Wasels, B.: Klin. Wchnschr. **7**:53, 106 and 153, 1928.
2. Roffo, A. H.: Ztschr. f. Krebsforsch. **37**:1, 1932.
3. Goldfeder, A.: Ztschr. f. Krebsforsch. **39**:421, 1933.
4. Warburg, O.: Ueber dem Stoffwechsel der Tumoren, Berlin, Julius Springer, 1926.
5. Braunstein, A.: Deutsche med. Wchnschr. **4**:880, 1923.

could be used in this manner, tetramethylarsonium was chosen because of its low general toxicity and, in part, because of its relative ease of preparation.

EXPERIMENTS

Preparation of Agents.—Tetramethylarsonium iodide was prepared by Cahours' method.[6] Arsenic trioxide and methyl iodide were heated in sealed bomb tubes to a temperature of from 165 to 175 C. for from twenty to twenty-four hours; after recovery of the unused methyl iodide by distillation, the residue was dissolved in strong caustic alkali, which was neutralized with concentrated hydrochloric acid. After allowing time for the separation of arsenic trioxide, this was filtered off, and the solution was evaporated to dryness on a water bath. From this product the arsonium halides were separated by successive solution in hot and freshly dehydrated ethyl and butyl alcohols. The residue left from evaporation of the latter solvent was dissolved in water and treated with an excess of silver oxide until the filtrate no longer reacted for halides, and the tetramethylarsonium hydroxide so obtained was purified by filtration and recrystallization.

Two acids derived from dextrose were used. Gluconic acid was obtained by the addition to a solution of pharmaceutic calcium gluconate of an equivalent quantity of oxalic acid, the precipitated calcium oxalate being removed by centrifugation. Qualitative tests showed traces of calcium but no oxalic acid. Glycuronic acid was prepared by the method of Quick[7] from the urine of dogs fed borneol. With both these acids and with one other the tetramethylarsonium salts were obtained by the addition to their solutions of the arsonium hydroxide to a point at which the solutions were neutral to litmus. None of these salts was obtained in crystalline form.

The lead compounds were prepared rather simply. The respective acids or their lactones were added to a suspension of an excess of lead carbonate, and the mixture was heated to boiling. The excess of undissolved carbonate was then removed by centrifugation. In addition to lead gluconate and glycuronate, salts were prepared from a number of miscellaneous sugar acids which were furnished through the cooperation of Dr. Fred Upson of the department of chemistry of the University of Nebraska. Of these, only one was tested in the form of the arsonium salt.

Tumors.—Transferable rat tumors, obtained for the most part through Dr. Francis Carter Wood of Crocker Institute, Columbia University, were used entirely for this work. In the earlier stages of the work studies were made on individual rats, but it developed quickly that, with respect to the agents which had any evident effect, the tumors came into two categories—slightly malignant ones, which yielded with some readiness to treatment, and others, more readily inoculable as a rule and more rapid in growth, with which results were obtained with more difficulty. Two representatives of this type were selected for the continuation of the work, which was then done with groups of rats handled similarly. These two tumors were the F R C carcinoma and the R39 rat sarcoma.

Procedure.—The agent, dissolved in physiologic solution of sodium chloride or, in later experiments, in distilled water, was injected intravenously through one of the caudal veins. The usual volume injected was 0.5 cc.; occasionally, with larger doses or in the case of relatively insoluble lead salts, 1 cc. was given.

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6. Cahours, A.: Ann. d. chem. **122**:192, 1862.
7. Quick, A. J.: J. Biol. Chem. **74**:331, 1927.

Lead Salts.—Although work was first done on arsonium compounds, the results obtained with salts of lead are here presented first, in tabular form. As may be seen, with the exception of two or three of these, they are not particularly noteworthy; in spite of what would be in man a proportionately altogether excessive dosage, destruction of tumor was observed only occasionally, except with lead glycuronate, lead galactonate and, somewhat less strikingly, with lead glucoheptonate. On the basis of these results, tetramethylarsonium glycuronate and galactonate were used for further study. That the general lack of results with lead compounds may be due to precipitation of the lead in the serum is probable; however, test tube experiments indicated that such precipitation was slow, usually requiring forty-eight hours for completion.

Tetramethylarsonium Salts.—Gluconate: The initial test of this attempted method of therapy was made with tetramethylarsonium gluconate with a tumor derived in this laboratory, of slow growth and rather difficult transmissibility.

Effects of Intravenously Injected Lead Salts on Implanted Rat Tumors

Salts	Dose, Mg.	Tumor	No. of Rats	Size of Tumor	Results
Gluconate.....	5	FRC	4	From 3 to 8 mm. in diameter	Complete disappearance of 1
Gluconate.....	8	FRC	1	1.5 by 2 cm. in diameter	No effect
Gluconate.....	5	FRC	4	Palpable*	Complete disappearance of 2
Gluconate.....	5	R39	5	Palpable	Complete disappearance of 1
Gluconate.....	5	R39	4	From 1 to 3 mm. in diameter	Complete disappearance of 1
Glycuronate.....	5	FRC	4	Palpable*	Complete disappearance of all
Glycuronate.....	5	R39	4	Palpable*	Complete disappearance of 2
Arabonate.....	4.3	FRC	3	Palpable*	Complete disappearance of 1
Arabonate.....	4.3	R39	4	Palpable*	Complete disappearance of 1
Galactonate.....	4.1	FRC	4	Palpable*	Complete disappearance of all
Galactonate.....	4.1	R39	4	Palpable*	Complete disappearance of 2
Glucoheptonate..	5	FRC	4	Palpable*	Complete disappearance of all
Glucoheptonate..	5	R39	4	Palpable*	Complete disappearance of 1
d-Mannonate.....	5	FRC	4	Palpable*	No effect
d-Mannonate.....	5	R39	3	Palpable*	Complete disappearance of 1
Rhamnohexonate	5	FRC	4	Palpable*	No effect
Rhamnohexonate	5	R39	4	Palpable*	No effect

* These tumors were treated three days after implantation.

Only one of these tumors was available at the time of this test, but this was used, as no particular interest attached to its indefinite propagation, and there was no great expectation of a positive result. The tumor was 0.5 cm. in diameter. On the day following the injection of 5 mg. of the salt the tumor showed definite softening, and after palpation the animal became extremely intoxicated, with prostration and slow and shallow respiration—a degree of intoxication that was never observed subsequently, possibly because since then early palpation was avoided. Four days after the injection the tumor was represented by a boggy mass, and after eleven days it had completely disappeared. When the animal died of intercurrent disease about six months later the site of the tumor was represented by a small pigmented area of infiltrated leukocytes, principally mononuclear.

Three rats with tumors of strain J R S were given injections of 5 mg. of the arsonium salt; the tumors were 0.5 cm. in diameter. In two the tumors had completely disappeared within about three weeks; in the other there was temporary softening and shrinking, but later resumption and continuation of growth. Two rats with large tumors, 2 and 2.5 cm. in diameter, respectively, were similarly treated, without effect.

Only larger tumors of strain 256 were treated; these were from 1 to 2 cm. in diameter. Five milligrams was given to each of three rats, without effect. Two animals with tumors initially 1 and 1.5 cm. in diameter, respectively, received daily injections of 5 mg. of the arsonium gluconate for eight days; there was no apparent effect.

Eleven rats with tumors of strain F R C from 0.5 to 1 cm. in diameter were initially given single doses of 5 mg. of the tetramethylarsonium salt. In five the tumors had completely disappeared within three weeks. Of the others, one was again given an injection of the same dose after a four day interval, without effect. Another was given a second injection of 20 mg. after a three day interval. This animal died after six days, with progressive shrinkage of the tumor in the interim. Another animal which was similarly treated showed temporary softening and shrinking of the tumor, but later progression. One of the rats with a tumor initially 0.75 cm. in diameter received a daily injection of 5 mg. of the arsonium salt, beginning four days after the first injection. A total of nine injections was given. The tumor continued to increase in size, although when the animal was killed a month later it was almost wholly necrotic, measuring at this time 3 by 2 by 1.5 cm. Two animals were used for experiments with insulin, which will be described later. One other rat, with a tumor 1 cm. in diameter, was given a single injection of 30 mg. It died forty-five days later, with an almost wholly necrotic tumor measuring 1 by 1.5 by 2 cm.

Of ten rats in which tumors of strain R39 had been implanted three days previously and which received injections of 5 mg. of the arsonium gluconate, four showed complete disappearance of the tumors within three weeks. The others showed continued growth of the tumor.

Four rats with older tumors of delayed growth and of small size (from 1 to 5 mm. in diameter) showed no effects from a dose of 5 mg.

Two rats with larger tumors, 1.5 by 1.25 cm., which were given successive doses of 5 mg. and 20 mg. of the arsonium gluconate after intervals of two and four days, respectively, failed to show any effect on the tumors.

Glycuronate: With early tumors of strain F R C of three days' implantation disappearance of the palpable masses was observed in only three of ten animals after the injection of 5 mg. of tetramethylarsonium glycuronate. Larger tumors, from 0.5 to 1 cm. in diameter, which were treated by the injection of 10 mg., likewise reacted only occasionally to the treatment, progression taking place in all except two of the ten animals.

No effect was observable in four animals with three day tumors of strain R39 treated by the injection of 5 mg. of arsonium glycuronate. Another series of four animals with tumors 0.5 cm. in diameter received a dose of 10 mg., also without effect.

Galactonate: Eight rats with tumors of strain F R C, which had been implanted five days previously and had an average diameter of 0.5 cm., were given an injection of 5 mg. of tetramethylarsonium galactonate. All showed continued growth of the tumor.

Nine rats with tumors of strain R39 of three days' implantation received 5 mg. of the arsonium galactonate. The tumors were palpable in all the animals, and all showed progressive growth.

Arsonium Salts and Insulin.—In general, it must be stated that treatment of the more malignant tumors by arsonium salts of sugar acids failed to give particularly striking results. However, with the gluconate, disappearance of the tumors was observed with sufficient frequency and with a reaction so apparently immediate as to suggest an occasional selective affinity for the toxic agent—an

affinity that appeared to be more constantly present with some of the less malignant tumors used in the earlier work. Since it seems safe to assume that the agent after absorption should have approximately equal toxicity for cells of the several strains of tumor, it would appear that selective absorption is frequently lacking with the more malignant tumors. On the basis of the possibility that this inconstant lack of absorption was due to greater affinity of the tumor cells for carbohydrate, as a result of which this immediately available substance could serve to shield the tumor cells from its toxic modification, the next series of experiments was carried out in circumstances in which the available supply of carbohydrate was temporarily reduced by the simultaneous administration of insulin. Large but not intoxicating doses were used. The rat appears to tolerate insulin better than man, and in preliminary experiments it was found that 0.08 unit could be given to rats weighing slightly more than 100 Gm. without evidence of intoxication. In the course of the experiments this dose was occasionally given, and half this dose was frequently given, to rats weighing 80 Gm., without eliciting symptoms of hypoglycemia.

Gluconate and Insulin: Twelve rats with three day tumors of strain F R C were given injections of 5 mg. of tetramethylarsonium gluconate and 0.04 unit of insulin. In four there was evident softening of the tumors within four days and complete disappearance a week later. At that time the remaining rats, in which the tumors were now 0.5 cm. in diameter, were given 5 mg. of the arsonium salt alone. In five of these animals complete disappearance of the tumors followed; progressive growth occurred in three.

Six rats with tumors which had been implanted ten days previously and which at the time of injection were slightly less than 1 cm. in diameter, were treated with 5 mg. of the arsonium salt and 0.04 unit of insulin. Nine days later the tumors had disappeared in five. The remaining rat was then given an injection of 5 mg. of the arsonium salt alone. The second injection was without effect.

In an experiment conducted along with the foregoing, six rats with tumors which were similar in every respect were given an injection of 5 mg. of the arsonium salt. Within nine days complete disappearance of the tumors had occurred in four. The other two were then given an injection of 5 mg. of arsonium salt and 0.04 unit of insulin. Here, as in the preceding series, the second injection was without effect.

In two series of experiments, lots of twelve and ten rats with three day tumors of strain R39 were given an injection of 5 mg. of arsonium salt and 0.04 unit of insulin. In the first series, complete disappearance of the tumors occurred in all within about two weeks; in the second, complete disappearance occurred in all except one rat within the same time. The last animal showed progressive growth of the tumor.

Larger tumors failed to react to treatment: Twelve rats with tumors which had been implanted twelve days previously and which averaged about 1 cm. in diameter were given an injection of 20 mg. of arsonium salt and 0.08 unit of insulin. One of these rats died three days later, with definite evidence of hepatic intoxication. Continued growth of the tumors followed in ten rats, only one showing disappearance of the tumor.

Twelve rats with tumors which had been implanted seven days previously received 5 mg. of arsonium salt and 0.04 unit of insulin. Six of these animals showed definite growth of the tumors a week later; the other six showed doubtful growth. The animals were then given a second injection of 20 mg. of arsonium salt and 0.08 unit of insulin. There was disappearance of the tumors in four of these animals.

Glycuronate and Insulin: As arsonium glycuronate appeared less effective when used alone than the gluconate did, its combination with insulin was tried only with tumors of strain R39. Twelve rats with three day tumors were given 5 mg. of the arsonium salt and 0.04 unit of insulin. A week later none of the tumors showed softening, and most gave evidence of progressive growth. These animals were then given a second injection, half receiving 10 mg. of arsonium galactonate and half 10 mg. of the galactonate and 0.04 unit of insulin. The tumor continued to grow in all the rats except one, in which it disappeared.

Galactonate and Insulin: Eight rats with tumors of strain F R C, which had been implanted five days previously, were given an injection of 5 mg. of tetramethylarsonium galactonate and 0.04 unit of insulin. All showed progressive growth of the tumors.

Nine rats with tumors of strain R39, which had been implanted three days before, were given an injection of 5 mg. of the arsonium galactonate and 0.04 unit of insulin. There was disappearance of one tumor only; the others showed progressive growth.

Insulin Alone: Ten rats in which tumors of strain R39 had been implanted three days previously were given an injection of 0.04 unit of insulin. Uninterrupted growth of the tumors occurred in nine animals, with disappearance of the tumor in one.

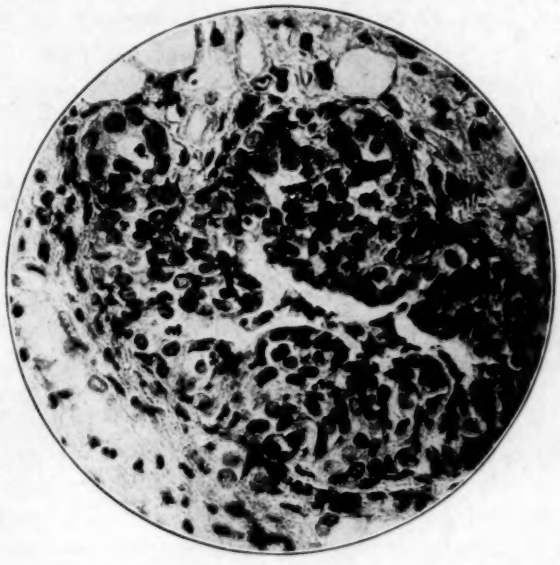
COMMENT

The two tumors which were used for the greater part of this work must both be regarded as of rather high virulence. Of the two, strain R39 was decidedly of greater malignancy than strain F R C; it withstood implantation with practically complete success and was of very rapid growth, killing its host generally within about seven weeks. Strain F R C also withstood implantation well, but not with the uniform success observed with strain R39; it was of slower growth, and took considerably longer to cause death. These differences in behavior are reflected in the reactions of these tumors to the agents used in this study.

Several lead salts were found to cause complete disappearance of all the F R C tumors treated; with strain R39 the best achievement was the disappearance of 50 per cent of the small number of tumors studied. It was hoped that the response of these tumors would afford a guide in the selection of the best acid radical to be used with the tetramethylarsonium base. This was not the case, and results obtained with lead salts gave no clue as to the effectiveness of the arsonium compounds. It is possible that had the former been used in conjunction with insulin, an apparent relationship might have become manifest. But the best results obtained with the lead salts, with a relative dosage that would be utterly unsafe for a human being, lagged so far behind those obtained with arsonium gluconate that it was judged inexpedient to follow their investigation further.

Of the several arsonium salts studied, conspicuous success was obtained with one only—tetramethylarsonium gluconate. With this alone it was possible to cause the disappearance of relatively non-

malignant tumors in the small number of rats studied with some, but not absolute, regularity. With more malignant tumors this effect was occasionally manifest if the tumors were treated while still small. A much greater uniformity of success was obtained with these tumors, if small, when the arsonium salt was administered along with insulin. The fact that this procedure was more uniformly successful with the more malignant R39 tumor than with the F R C strain accords with the theory on which the coadministration of insulin is based. If the affinity of a cancer cell for carbohydrate is a function of its malignancy, or vice versa, with less malignant tumors less ability of the cancerous



Single tumor colony three days after implantation; numbers of these colonies appeared in the tissue encapsulating the embedded tumor mass, which was almost wholly necrotic.

tissue to avail itself of systemic reserve carbohydrate and so less chance of a shielding effect by this might be expected.

With strain R39, particularly, the only tumors treated with any uniformity of success were those which had been implanted a short time (three days) previously. Only rats with definitely palpable masses at this time were used. That actual implantation had occurred within this limited time is indicated by the photomicrograph, which shows one of a number of isolated tumor colonies in the peripheral tissue around an implanted fragment. The latter showed apparently complete necrosis. There has not been as yet an opportunity to determine the exact degree of progress that can be permitted these tumors

before successful treatment is no longer possible. As the results indicated, this is greater for strain F R C than for strain R39.

The toxicity of tetramethylarsonium gluconate for rats is low. While occasionally animals died comparatively shortly after receiving a 5 mg. dose, these deaths occurred so seldom that they must be regarded as due to intercurrent disease. The intoxication observed after this dose was acute and apparently entirely associated with disintegration of the tumor. Nor was early death observed after the fewer doses of 10 mg. A number of premature deaths followed a 20 mg. dose; the single animal that received 30 mg. died forty-five days later. When the gluconate was given in doses of 5 mg. on successive days, several animals received from 40 to 45 mg. without evidence of intoxication. Only one animal was observed with changes of tissue indicative of death from intoxication—a rat weighing about 80 Gm., which had received 20 mg. of the arsonium gluconate along with 0.08 unit of insulin; this was the only one of twelve animals of this series to succumb.

In the interests of brevity, no detailed account has been given of the course of the treated tumors. In general, disappearance of the tumor was preceded by a period of definite softening, with gradual absorption of the softened mass. At times there was direct shrinkage, with increased induration; recurrences were more likely to occur after this than after softening, though they occurred occasionally also with the latter. The relation of the disappearance of these tumors to the therapeutic procedure may perhaps be questioned, in view of the fact that spontaneous disappearance of implanted rat tumors is occasionally observed. In this work practically all the inoculated rats were subjected to some form of therapeutics, and the rate of natural disappearance, which is at best variable, but low, was not observed. Indirect evidence to the effect that treatment was responsible for the disappearance is furnished by the peculiar course of the latter—the rather prompt softening, followed by gradual absorption. Also, the relatively high rates of disappearance in the suitably treated rats were altogether disproportionate to those in animals treated otherwise.

There has been no opportunity to try the effects of combined arsonium salt and insulin therapy on native tumors. Several unsuccessful attempts were made to produce tar cancers in rabbits for this purpose, with tar obtained from the local gas-works. The tar appeared to be more toxic than cancerogenic, as all the animals died before or during the stage of benign papillomatosis. As to tumors in man, these would only rarely be suited to experimental trial. For this purpose it would appear necessary to select cases of early and superficially accessible recurrent malignant growth; late and otherwise hopeless cases would be useless, in view of the quantitative relation shown so clearly

with rat tumors. For the same reason, should the agent show any effectiveness with tumors in man, its use would appear to be as an adjunct to surgical removal of the great mass of the tumor tissue.

SUMMARY

On the basis of the theoretical possibility of effecting selective intoxication of cancerous tissue by means of carbohydrate compounds of toxic character, a study was made of the effects on transplantable rat tumors of various agents of this sort.

The toxic radical was represented by lead and tetramethylarsonium, the carbohydrate by a number of acids derived from hexose.

Results with the lead compounds were, in general, unsatisfactory; while it was possible to cause the disappearance of a number of implanted tumors of relatively low malignancy, with the most malignant tumor studied disappearance, on the whole, occurred only occasionally.

With the tetramethylarsonium compounds, the gluconate alone, when given in an apparently safe dose, caused the disappearance of a considerable proportion of a limited number of tumors of low virulence. With more malignant tumors, more effective results were obtained by the coadministration of insulin. When this treatment was used with the most malignant tumor studied, disappearance of the tumor followed in twenty-one of twenty-two cases.

If chemotherapy is to be effective, early treatment seems necessary. There appears to be a definite quantitative relationship between the effectiveness of treatment and the progress of the tumor, which has not been worked out in detail.

THE RÔLE OF HISTAMINE IN INFLAMMATION

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The substance β-iminazolylethylamine, which is readily formed by the decarboxilation of histidine, has been given the name of histamine. This chemical material was first discovered by Yoshimura [1] in 1909, while in the following year Ackerman [2] produced it by allowing bacteria to act on the amino-acid histidine, and Barger and Dale [3] isolated it from ergot. In a recent communication, Best and McHenry [4] presented a detailed account of the many sources of this chemical body, and it is now concluded that histamine is a normal constituent of all body cells.

During the first few years following its discovery, the method of action and the effects of the drug were chiefly of physiologic interest. It was soon found, however, that there appeared to be a definite relationship between the effects of large doses of histamine, anaphylactic shock in animals and secondary wound shock in man. This discovery suggested the possibility that the amine in question, or some substance closely related to it, was either liberated or produced by the body under certain pathologic conditions. The work of Sir Thomas Lewis [5] definitely linked histamine to the study of physiology, pathology, immunology, dermatology and general medicine.

Cohnheim [6] was the first investigator to present the hypothesis that the vascular reaction is the factor of prime importance in an area of inflammation. In the monograph by Adami,[7] this question is discussed fully, but the process by which the phenomenon occurs is not explained. More recent work has suggested the possibility that the alteration in vascular structure and permeability which occurs at the site of an inflammatory lesion may be due to the effect of one or more of the products of cellular disintegration caused by the inflammatory agent. Since it is known that histamine is a normal constituent of all tissues and that it produces a vascular reaction similar to that observed in

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1. Yoshimura, K.: Biochem. Ztschr. **28**:16, 1910.
2. Ackerman, D.: Ztschr. f. physiol. Chem. **65**:504, 1910.
3. Barger, G., and Dale, H. H.: J. Physiol. **40**:38, 1910.
4. Best, C. H., and McHenry, E. W.: Physiol. Rev. **11**:371, 1931.
5. Lewis, Thomas: The Blood Vessels of the Human Skin and Their Responses, London, Shaw & Sons, 1927.
6. Cohnheim, J.: Lectures on General Pathology, translated from second German edition by A. B. McKee, London, The New Sydenham Society, 1889.
7. Adami, J. G.: Inflammation, London, Macmillan and Company, Ltd., 1909.

inflammation, it has been suggested that this reaction is secondary to the liberation of the amine, irrespective of the type of irritant causing the inflammation.

Eppinger [8] described the reaction of the vessels of the skin to histamine as consisting of a threefold response: (*a*) a primary and local dilatation of the minute vessels of the skin, (*b*) a widespread dilatation of the neighboring strong arterioles, brought about entirely through a local nervous reflex, and, locally, (*c*) increased permeability of the walls of the vessels.

In his monograph, Lewis [9] demonstrated that the same type of inflammatory reaction can be produced by mechanical, electrical, thermal and other stimuli and concluded that the vascular changes are due to the effects of histamine or an H-substance liberated from the cells so stimulated. Dale [10] has drawn attention to the fact that the locus of the action of the amine on the vascular system shifts to a more proximal point as the biologic tree is ascended. The effects of histamine on the vessels of the dog, monkey and man have been studied by Burn and Dale [11] and others, who have concluded that in these species the amine produces a capillary and arteriolar dilatation and a constriction of the arteries and veins. Oertel,[12] in a recent publication, agreed with Ricker [13] that inflammatory hyperemia does not occur through a greater flux of blood and stated that this congestion is secondary to an arterial constriction. Though Oertel did not mention histamine, he appeared to be describing the same vascular state as that produced by this drug.

The early phenomena which occur in the inflammatory process, irrespective of the type of irritant, and which are dependent on the vascular reaction of the tissue, consist of: (*a*) dilatation and engorgement of the minute vessels, (*b*) exudation of fluid, (*c*) diapedesis of leukocytes and erythrocytes and (*d*) the formation of fibrin and an attempt to wall off the inflammatory focus. It therefore follows that if histamine can incite a true inflammatory response, it will reproduce all of the foregoing phenomena.

Eppinger, Lewis and others proved that the application of the amine to the cutaneous vascular system of dog and man results in a dilatation of the capillaries and arterioles and an exudation of fluid. Bloom,[14] Paul,[15] and Grant and Wood [16] reported that they were unable to show

8. Eppinger, H.: Wien. med. Wchnschr. **43**:1414, 1913.
9. Lewis,[5] p. 235.
10. Dale, H. H.: Lancet **1**:1179, 1233 and 1285, 1929.
11. Burn, J. H., and Dale, H. H.: J. Physiol. **61**:185, 1926.
12. Oertel, H.: Canad. M. A. J. **29**:378, 1933.
13. Ricker, G.: Frankfurt. Ztschr. f. Path. **33**:45, 1926.
14. Bloom, W.: Bull. Johns Hopkins Hosp. **33**:185, 1922.
15. Paul, J. R.: Bull. Johns Hopkins Hosp. **32**:20, 1921.
16. Grant, R. R., and Wood, J. E.: J. Path. & Bact. **31**:1, 1928.

that histamine was positively chemotactic. Wolf,[17] on the other hand, stated that the amine is strongly chemotactic in vivo and in vitro, and Weiss and his co-workers [18] produced leukocytosis by continuous intravenous injections of histamine in man. The question of the formation of fibrin about an area of reaction produced by histamine has received little or no notice. The recent work of Menkin [19] demonstrated that irrespective of the etiologic factor of its production a network of fibrin, occluding the intercellular spaces and the lymphatic channels, is formed about an inflammatory focus. On the suggestion of Professor Klotz, experiments were performed in an attempt to determine whether histamine is an agent calling forth all the characteristics of the inflammatory reaction and the manner in which it brings about its effect.

MATERIALS AND METHODS

All solutions used in the experiments consisted of histamine acid phosphate in physiologic solution of sodium chloride. Since solutions of histamine rapidly lose strength unless sterile (Best and McHenry [4]), all solutions were put in rubber-capped vaccine bottles and sterilized in the water bath. Tests for sterility and potency were made frequently.

In many of the experiments the capillaries of the skin were examined microscopically, and it was found that though only the minute, superficial vessels could be seen, with practice it was possible to observe accurately the changes which occurred in these structures. The skin was illuminated by a high power lamp, its rays being concentrated by lenses into a brilliant point of light. This light was filtered through a water cell containing a few drops of methylene blue. The filter, which cut out the heat rays from one end of the spectrum and the ultraviolet rays from the other, served to render the vascular structures more distinct as well as to protect the skin from the heat. A freely mobile, binocular, dissecting microscope was used. When practical, a drop of cedar oil was placed on the area to be examined, with a resultant marked increase in the clarity of the structures.

During the course of the experiments every care was taken to insure sterility of solutions, instruments and skin, so that the results obtained would not be complicated by infection. Very fine hypodermic needles were used and carefully inserted into the tissues in order to reduce the factor of trauma to a minimum.

EXPERIMENTS

In this experimental work the guinea-pig, rabbit, dog and human being were used. Since the results obtained in the guinea-pig corresponded to those in the rabbit, only the latter will be described. The effects of histamine on the vascular structures of the latter species are minimal. It was possible to cause a moderate dilatation of the conjunctival vessels by direct application of the amine, but injection failed

17. Wolf, E. P.: J. Exper. Med. **34**:375, 1921; ibid. **37**:511, 1923.

18. Weiss, S.; Robb, G. P., and Ellis, L. B.: Arch. Int. Med. **49**:360, 1932.

19. Menkin, V.: Arch. Path. **12**:802, 1931; Arch. Int. Med. **48**:249, 1931; J. Exper. Med. **56**:157, 1932; Proc. Soc. Exper. Biol. & Med. **30**: 1069, 1933; J. Exper. Med. **57**:977, 1933.

to produce a wheal or a flare of the skin. In this work an attempt was made to produce an inflammatory reaction in the rabbit by applying histamine by various methods. The conclusion was reached that the amine, when repeatedly instilled into the conjunctival sac, applied to the intact, burned, scratched or cut skin for as long as seventy-two hours or repeatedly injected into the muscle of the rabbit, does not produce an inflammatory reaction.

The effect of histamine on human capillaries was investigated by performing the following experiment: A pneumatic cuff was placed about the arm and quickly pumped to above systolic pressure. This procedure rapidly produced a condition of circulatory stasis, and the microscopic appearance of the capillaries of the skin of the forearm was noted. A minute amount of histamine was injected intradermally, and there quickly appeared a small purplish spot at the site of the injection. Microscopic examination of this area showed an increase in number, size and tortuosity of the capillaries. This purplish spot did not spread so long as the pressure was maintained, but immediately after the pressure was released the spot extended rapidly and appeared as an irregular mulberry-colored area measuring about 1 cm. in diameter. This area showed a marked increase in number, size and tortuosity of the capillaries, with a sluggish flow of blood. The discoloration was transient, being rapidly obscured by the formation of a wheal which occurred at the site of the vascular dilatation. The wheal reached its maximum size in about five minutes, and the fluid was then gradually reabsorbed, so that it had entirely disappeared at the end of one hour.

The observations recorded suggest the following conclusions: The action of histamine on the minute vessels of the human skin produces a marked increase in number, size and tortuosity of the visible capillaries, with engorgement and a sluggish flow of blood through these structures. The release of pressure allows the amine to infiltrate the adjoining tissues and results in similar vascular changes. The formation of a wheal occurs at the site of vascular dilatation and is a result of serum exudation associated with the capillary damage caused by the amine. Ebbecke [20] showed that histamine produces a marked increase in permeability of the capillary wall during the period of wheal formation (about five minutes), and that at the end of this time the transudation ceases and a slow absorption of fluid commences. In his discussion on wheals, Lewis [21] stated that:

> The outpouring of fluid into the tissue spaces is not the result of an increased filtration pressure. The increased permeability is not the result of simple stretching of the vessel's wall; it is the result of an independent change in the wall in response to stimulation, whereby this wall becomes unusually pervious.

20. Ebbecke, U.: Klin. Wchnschr. **2**:1725, 1923.
21. Lewis,[5] p. 80.

By comparing a site of the injection of histamine and an area of known inflammation, it is seen that the vascular structures of the two are similar in appearance. In each of these regions there is an increase in the number and tortuosity of the visible capillaries. The vessels are markedly engorged; the flow of blood through them is slow, and, in addition, both areas show an increased permeability for fluid.

The next experiment was performed in an effort to determine the effect of histamine on the migration of leukocytes, and was carried out as follows: The back of a dog was shaved, and into each of four well separated areas 0.1 cc. of a solution of histamine (1 : 500) was injected. One area was widely excised at the end of five minutes, another in ten minutes, the third at the end of one hour and the fourth at the conclusion of eighteen hours. The tissues were immediately placed in Orth's solution,[22] but the wheal which was present in the first two sections could not be preserved. Sections of tissue from the block removed five minutes after the injection of the amine showed many dilated capillaries, which were well filled with blood cells. The collagen fibers of the subcutaneous tissue presented no notable change, though in some areas they were somewhat separated. It was difficult to recognize the pathway of the needle through the tissues, since it was represented by only a small area of torn and distorted collagen fibers. There were no leukocytes in the tissue, and no diapedesis of erythrocytes had occurred. Sections of tissue removed ten minutes after the injection of histamine presented exactly the same changes as noted previously. The third block of tissue was also similar to the first two, except that the visible capillaries were not so numerous and were not engorged and distended with blood cells.

In the section of skin removed at the end of eighteen hours there was a definite inflammatory response. The track of the needle was represented by a small area of degenerating collagenous material, in and about which were scattered polymorphonuclear leukocytes and lymphocytes. This inflammatory cell infiltration was, however, not limited to the tissue immediately surrounding the pathway of the needle but extended well out to the periphery of the section. The number of leukocytes in any one field was found to decrease as sections more distal from the point of injection were examined. The vascular response was moderate in degree, and no diapedesis of erythrocytes had occurred.

The results of this simple experiment presented some interesting points. It was noted that one hour after the injection of histamine into the skin, at which time the effects of the amine were almost entirely dissipated, there was no cellular response. Though histamine had produced a vascular dilatation and exudation of fluid, at the end of one

22. Orth's solution consists of: bichromate of potassium, from 2 to 2.5 Gm.; water, 100 cc., and formaldehyde (40 per cent solution), 10 cc.

hour the sections of tissue presented no evidence to substantiate a hypothesis that the amine had a positively chemotactic force. Histamine infiltrates the tissues through the tissue and lymphatic spaces and produces the foregoing vascular reaction in all capillaries with which it comes in direct contact. The wheal begins at the point of injection and quickly follows the capillary dilatation. Subsequently, the fluid exudate, combined with the drainage of the amine through the blood and lymph stream, decreases the concentration of histamine in the tissues to a point at which it is no longer able to produce its vascular reaction. A fairly large dose of a strong solution of the drug was used, and it is apparent that the tissues immediately about the point of injection were subjected to a greater concentration of the solution of histamine for a longer period of time than were the more distant tissues; also, that both the strength of the solution and the time of action decreased toward the periphery of the wheal. In addition, the injection of the solution had two direct effects, the first being traumatization of the cells along the pathway of the needle and the second the rapid distention of the region by the exudation of fluid, with its resultant tearing of tissue and cell destruction. The production of the wheal served to cause further damage to the tissues, though since its formation was more gradual the effects were probably of a lesser degree.

The presence of the cellular infiltration in the section of tissue removed at the end of eighteen hours indicates that there was present in the tissues some substance which was positively chemotactic. The following possible sources of this attraxin must be considered: (*a*) a chemotactic substance liberated from the cells injured by the needle, (*b*) the histamine solution, (*c*) a product of cellular disintegration resulting from the direct action of histamine on tissues or (*d*) a product of cell destruction caused by the edema of tissues.

Histamine was injected into the shaved skin of a dog, and sterile needle punctures were made into several areas, both in and outside the flare. At the end of eighteen hours sections of skin were removed. The tissue into which histamine was injected presented the microscopic picture previously described, whereas the areas into which sterile needle punctures were made showed only a few scattered leukocytes about the wound. This reaction was not influenced by the presence of a flare. The experiment proves, therefore, that the chemotactic action of the products of cellular destruction caused by needling is practically negligible.

The next possible source of the chemotactic substance to be considered was histamine itself. It has been noted that there was no diapedesis of leukocytes after histamine had been allowed to act on the tissues and vascular structures for one hour. During this period the amine had been undergoing dilution and absorption, so that at the conclusion of the hour the effects of the drug had almost entirely dis-

appeared. By removing the skin of sterile blebs, produced by burning, it was possible to examine directly the vessels of the skin and to observe the effect of the direct application of the amine to these structures. The denuded areas were flooded with solutions containing various amounts of histamine, and it was possible to secure the aforementioned effects of the drug on the visible capillaries. Though the vascular dilatation and engorgement were continued for two hours, it was not possible to demonstrate any migration of leukocytes from the vessels to the fluid.

From the results of the foregoing work the conclusion was reached that the chemotactic substance in question was a product of cellular destruction. The cause of this damage to the tissue was due either to the effect of histamine on the cells or to the trauma to the tissue caused by the production of the wheals. To investigate this question, a wheal was produced by the injection of histamine into the skin of a dog's back, and in another area sufficient physiologic solution of sodium chloride to produce a similar-sized swelling was slowly injected. There was one factor which complicated this work and which it was impossible to obviate, namely, the liberation of histamine from the cells damaged by the injection of saline solution. However, this amount of histamine was very small, since it did not cause either a further wheal at the site or a surrounding flare. Since there was a large amount of fluid already present, any histamine liberated was greatly diluted, and this dilution was sufficient to prevent the appearance of any appreciable reaction to histamine.

Microscopic examination of the sections failed to demonstrate any difference between the areas into which the saline solution was injected and the areas in which a wheal was produced by histamine. Both tissues showed a diffuse infiltration of leukocytes with a slight concentration of white cells about the point of injection. This work shows that at the end of eighteen hours the cellular migration into an area of traumatized tissue, as produced by an injection of saline, was equal in degree to that which occurred as the result of an intracutaneous injection of histamine. In both of these areas there was present one common factor, namely, a rapid increase of fluid in a localized area of cutaneous tissue. This fluid caused tearing and distortion of the cellular structures, with the liberation of the products of tissue disintegration.

All microscopic sections were carefully examined to determine the amount of fibrin present. It was found that there was only an occasional strand of fibrin about the site of the injection of histamine and that there was just as much of this material at the site of the injection of saline solution. Though the reactions were allowed to proceed for as long as thirty-six hours, the formation of a network of fibrin in the tissue or lymphatic spaces was not demonstrated. In view of Menkin's work on the subject of inflammation, this result is interpreted as show-

ing that the application of histamine does not play an important part in this phase of the inflammatory process. Although, as Lewis has shown, the wheal fluid consists largely of the constituents of blood plasma, it is evident that the other factors concerned in the formation of fibrin are not active.

COMMENT

The foregoing experiments were performed in an effort to determine the rôle played by histamine in the production of inflammatory reactions in the skin. The effect of the cellular destruction due to trauma by the needle may be practically disregarded, since the skin punctured by a fine sterile needle consistently shows only a few leukocytes about the injured tissues. Lewis and his co-workers showed that the vascular reaction about a needle prick is dependent on the liberation of histamine from the injured cells. Histamine is, however, only one of the products of cellular disintegration, and the fact that it produces certain vascular phenomena is not sufficient reason for assuming that it is also the substance which attracts leukocytes into the tissues. No leukocytes were noted in the tissues one hour after an injection of histamine, and it has been shown by others that at the end of this period there is little histamine in the area into which the injection was made. The direct application of the amine to the vessels of the skin failed to attract leukocytes from the blood vessels. The experiments cited seem to prove that histamine is not positively chemotactic. Since no evidence was obtained to substantiate the hypothesis that histamine is positively chemotactic, and since the cellular infiltration of tissues damaged by the injection of saline solution is equal to the number of white cells at the site of a wheal produced by the injection of histamine, it was concluded that this chemotactic substance is a product of cellular disintegration other than histamine. The failure of the histamine reaction in tissues to give rise to the formation of any appreciable amount of fibrin is important when the part played by the amine in the process of inflammation is considered.

CONCLUSIONS

Evidence has been presented to show that histamine, when acting on the minute vessels of the skin of dog and man, causes capillary dilatation and engorgement with a slowing of the blood stream and an exudation of fluid. Injury and destruction of tissue, irrespective of the etiologic factor, will allow the liberation of small quantities of the amine, resulting in this reaction. The rôle played by histamine in the process of inflammation is confined to its effect on the small vessels. Histamine does not call forth the cellular elements in the inflammatory exudate. Further work is required to determine the action of various other products of cellular disintegration.

Case Reports, Laboratory Methods and Technical Notes

MULTIPLE MALIGNANCY WITH METASTASIZING CARCINOID OF ILEUM AND MILIARY TUBERCULOSIS

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BOSTON

While we have shown [1] that multiple malignant tumors are far from rare, cases of triple malignancy are relatively infrequent; three such cases were recorded in our recent review, together with one newly reported case. We now report another, which is further noteworthy because of the coexistence of active miliary tuberculosis, claimed by Pearl [2] to be excessively rare in association with malignant disease, although Wilson [3] proved that Pearl's thesis is erroneous.

In addition, one of the three malignant tumors in this case was a metastasizing carcinoid. Raiford [4] found that carcinoid tumors represented only 0.18 per cent of gastro-intestinal tumors in his large series and that only one fifth had metastasized. Gaspar [5] summarized a few cases of metastasizing carcinoids of the small intestine and emphasized their rarity.

REPORT OF A CASE

History.—A. W., an unmarried white woman, aged 53, entered the Palmer Memorial Hospital in 1929 under the care of Dr. L. S. McKittrick, complaining of a painless abdominal mass of four months' duration. At operation, a malignant papillary adenocystoma of the left ovary was removed, together with peritoneal implants from the cecum, appendix and parietes, which microscopic examination showed to be metastases of the ovarian tumor. Following intensive high voltage roentgen therapy the patient recovered and remained fairly well for four years. In 1934 she reentered the hospital, under the care of Dr. R. H. Sweet, with a history of progressive weakness and cramplike abdominal pains of three weeks' duration. These symptoms were regarded as due to metastases of the previously removed tumor, and no active treatment was attempted. Five days after entry the patient died with evidence of chronic intestinal obstruction.

Autopsy.—The peritoneal cavity showed adhesions between the omentum and the anterior abdominal wall; the appendix was absent; the diaphragm reached the fourth rib on the right and the fifth rib on the left; the surfaces were slightly dull.

From the Laboratory of Pathology, New England Deaconess Hospital.

1. Warren, S., and Gates, O.: Am. J. Cancer **16**:1358, 1932.
2. Pearl, R.: Am. J. Hyg. **9**:97, 1929.
3. Wilson, E. B.: Am. J. Cancer **16**:227, 1932.
4. Raiford, T. S.: Am. J. Cancer **18**:803, 1933.
5. Gaspar, I.: Am. J. Path. **6**:515, 1930.

There was no free fluid or exudate. The sigmoid colon was dilated so that it extended almost up to the left costal margin and measured approximately 10 cm. in diameter. At the upper end of the dilatation the colon was twisted on itself, occluding the intestine, but without any surrounding inflammatory or degenerative reaction. There was an unusually long mesentery for the descending colon and sigmoid, which was twisted clockwise for nearly a complete revolution. The serosa of the lower dilated sigmoid was markedly congested. No mechanical obstruction was found in the pelvis. A few scattered firm nodules averaging 1 cm. in diameter were present in the broad ligament. The lymph nodes were palpable in the mesentery of the small intestine. Six firm, gray polypoid nodules, varying from 1.5 to 0.3 cm. in diameter, apparently arose from the mucosa of the ileum and descending colon. The sigmoid colon had a smooth stretched out mucosa in the upper portion and a congested dark red hemorrhagic mucosa with irregular foci of erosion in the lower sigmoid colon and in the rectum. Several thrombosed submucosal vessels were prominent. Except where ulcerated, this involved mucosa had a rather glassy turgid appearance. The right lung weighed 545 Gm.; the left, 520 Gm. In the left upper and lower lobes were a few scattered nodules of firm, glistening, semitransparent tissue which slightly everted on section, the largest being 1 cm. in diameter. In addition similar nodules about 0.2 cm. in diameter were scattered throughout the lung, giving it a sandy texture. No large nodules were found in the right lung, but there were similar fine nodules scattered throughout all the lobes. The suprarenal glands were normal except for a glistening white, firm, fairly discrete nodule 0.7 cm. in diameter at the tip of the right gland involving the medulla and extending into the cortex. The right kidney weighed 380 Gm.; the left, 275 Gm. The surfaces appeared smooth and pale when the capsules were stripped. The cortices were 0.6 cm. wide. In the tips of the pyramids were yellowish-white, smooth, irregular foci, the largest of which was 0.5 cm. in diameter. The pelves and ureters were normal. Involving the central portion of the right kidney was a cyst 5 cm. in diameter extending from the pelvis to 1.5 cm. beyond the surface of the kidney. It was filled with gelatinous yellow material. The parenchyma was slightly compressed around the cyst. The uterus was normal except for three very slightly raised, soft foci 0.6 cm. in diameter which extended 0.2 or 0.3 cm. below the surface. The bodies of the fourth and fifth lumbar vertebrae were white and contained foci of necrosis. White thick fluid raised the anterior periosteum; the cartilaginous disks were also invaded. The marrow of the upper lumbar vertebrae was dark red.

Microscopic Examination.—Scattered throughout both lungs were tubercles varying considerably in both size and age. The largest were caseous and had considerable surrounding fibrosis. Tubercles were found on the mucosal surface of many bronchi. In addition there was a focus of dense fibrous tissue with central calcification. Tubercles, chiefly early ones, were scattered throughout both the pulp and the corpuscles of the spleen. In the submucosa of the ileum there was a tumor nodule consisting of uniform, oval, medium-sized cells with no definite arrangement. These cells were not encapsulated and extended slightly into the mucosa and muscularis. Rare mitoses were present. Silver stain showed numerous argentaffin granules. In the colon the tubercles in the mucosa and submucosa did not show any definite ulceration. A polypoid mucosal nodule formed of closely packed, hypertrophied, atypical, mucosal glands invaded the muscularis. The cells showed mucous secretion, and mitoses were present. The sigmoid colon showed marked congestion and edema of all the coats with hemorrhage and necrosis of the mucosa. There was a diffuse infiltration of lymphocytes, plasma cells and polymorphonuclear leukocytes. Fibrin covered the serosal

surface. The liver was normal except for early tubercles in or near the majority of the portal spaces. The right suprarenal gland showed destruction of two thirds of the tissue by caseous necrosis with peripheral small tubercles, giant cells and fibrosis. Tubercles were scattered throughout the medulla of the kidney and a few scattered glomeruli were sclerotic. The uterus showed one small tubercle in the myometrium. The mesenteric lymph node was almost entirely replaced by tumor cells identical with those in the ileum. The hilar lymph nodes were replaced by dense fibrous tissue; there were a few scattered tubercles at the periphery. The bone marrow showed foci of caseous necrosis with peripheral giant cells and extensive destruction of the bone.

Anatomic Diagnosis.—The following diagnosis was made: resection of the ovaries for malignant papillary adenocystoma with peritoneal metastases; multiple carcinoid tumors of the small intestine with metastasis to the mesenteric lymph nodes; malignant adenoma of the colon; miliary tuberculosis of the lungs, spleen, suprarenal glands, kidney, myometrium and liver with tuberculosis of the colon, vertebrae and lymph nodes; torsion of the sigmoid colon with necrosis and hemorrhage; thrombosis of the superior hemorrhoidal artery; retention cyst of the right kidney; cholelithiasis and arteriosclerosis.

General Review

THE PROBLEM OF INTRANUCLEAR INCLUSIONS IN VIRUS DISEASES

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In trying to present this problem the German method of *Fragestellung* is used. The questions do not reveal information so much as they do the lack of it.

What are viruses?

The term "virus" is simply the Latin word for poison. Virus diseases are caused by substances whose organismal nature has not been proved. As soon as any virus is found to be a micro-organism it is no longer considered a member of the group. Some imagine viruses to be living agents, like those with which one is familiar, but smaller and more elusive. Others think they behave more like inanimate substances, such as enzymes. Still others regard viruses as living things of a type altogether new to present understanding. Be this as it may, viruses certainly occur in thousands in plants as well as animals. In attempting to learn something about them by the cellular responses which they provoke, my colleagues and I have concentrated attention on those which lead to the production of intranuclear inclusions.

What are inclusions?

Cytologists and virologists employ the word "inclusion" differently. By the former it is commonly applied to nuclear and cytoplasmic bodies that have nothing to do with viruses. According to Maximow and Bloom,[1] cytoplasmic inclusions comprise: "accumulations of proteins, fats, carbohydrates, pigments, secretory granules, chromophile substance and crystals." They are often looked on as "passive, lifeless, temporary constituents of the cell." Nuclear inclusions are of the same order. Reference may be made to fat droplets, crystals and melanin.

Virologists have simply appropriated the word "inclusion." Their original idea was that the inclusion material was of extracellular origin

From the Anatomical Laboratory, Washington University.

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1. Maximow, A., and Bloom, W.: Textbook of Histology, Philadelphia, W. B. Saunders Company, 1931.

—a view not borne out by recent work. In order to avoid ambiguity some now refer to the inclusions in question as "virus inclusions," which is satisfactory provided that it does not carry the impression that the inclusions are made of virus. It is more correct to speak of "inclusions in virus diseases." To do so, however, is not practicable because the phrase is cumbersome. In characterizing nuclear inclusions it is customary to emphasize: (1) the acidophilic staining of the inclusion mass, (2) the presence of a clear halo between it and the nuclear membrane and (3) the margination of basophilic chromatin on the nuclear membrane.

In what conditions are inclusions found?

It would be tedious to list each and every condition separately. There is, however, a range from severe epidemic diseases, like yellow fever, smallpox and many others, to mild infections called "inapparent" because their presence is not revealed by noticeable clinical symptoms, as the salivary gland disease of guinea-pigs.

Nuclear inclusions have been observed almost by chance in occasional individuals of all species whose tissues have been repeatedly examined: man, monkeys, dogs, guinea-pigs, rats and mice. Whether in each case their presence indicates virus action is uncertain. Since the occurrence of nuclear inclusions without signs of disease is just as important, in a statement of the whole problem, as their presence in disease so devastating that they irresistibly attract attention, an attempt has been made to secure data bearing on their distribution in wild animals also.

E. J. and L. E. Rector [2] discovered that all of fourteen common garden moles collected near St. Louis exhibited nuclear inclusions in their salivary glands. These proved to resemble closely the well known inclusions in guinea-pigs, young children and rats. There is every reason to suppose that further investigations will bring to light similar modifications in other species, likewise in the absence of revealing clinical symptoms.

I decided to go further, in cooperation with the Philadelphia Zoological Society. Dr. Fox, the pathologist in charge, Dr. Lucas and I have just completed a survey of many mammals and birds with special reference to the kidneys.[3] Nuclear inclusions something like those caused by the virus of yellow fever were seen in the kidneys of several parrot-like birds of Central America, termed "Guatemalan

2. Rector, Eleanor J., and Rector, L. E.: Intranuclear Inclusions in the Salivary Glands of Moles, Am. J. Path. **10**:629, 1934.

3. Fox, H.; Cowdry, E. V., and Lucas, A. M.: The Distribution of Intranuclear Inclusions Suggestive of Virus Action in Wild Animals, Am. J. Path., to be published.

amazons;" but they were never so frequent per unit volume of tissue as the yellow fever inclusions, nor were they accompanied by the same severe tissue reaction. Other inclusions, also belonging to type *A* (see following question), were found, while inclusions of type *B* were frequently encountered. That the latter are all due to virus action is questionable.

It is likely that nuclear inclusions are widely distributed in vertebrates with, but more often without, evidence of disease. The nuclear inclusions in polyhedral virus diseases of insects are interesting but atypical.

Can inclusions be classified in groups?

The reactivity of the nucleus is limited by the cytoplasmic buffer interposed between it and the environment and by the relative uniformity of the chemical substances of which nuclei are made and from which the reaction products must be built, unless outside materials enter and contribute to the formation of the inclusions, for which there is no evidence except in regard to water. Yet nuclear inclusions are not all alike by any means. Since it is risky to try to classify them on the basis of published descriptions, only those under investigation in this laboratory, or in preparations graciously sent to me by other workers, are considered.

Type *A* inclusions occur in:

1. Herpes
2. Yellow fever
3. Chickenpox
4. Virus III infection
5. Salivary gland disease of guinea-pigs
6. Salivary glands of moles
7. Kidneys of Guatemalan amazons
8. Fox encephalitis (from R. G. Green)
9. Whooping cough (from H. A. McCordock)
10. Salivary glands of rats (from Juanita Thompson)
11. Salivary glands of children (from S. B. Wolbach)
12. Infectious tracheitis of chickens (from Oskar Seifried)
13. A disease of parrots and parrakeets (from J. R. Meyer)
14. Kidneys of frogs (from Baldwin Lucké)
15. Brain of mouse with louping ill (from L. T. Webster)
16. Mad itch in dogs (from E. W. Hurst)
17. A disease of owls (from R. G. Green)
18. Many species in the absence of disease

The nuclear reaction is total and proceeds to complete degeneration. The inclusions are amorphous or particulate, but may be condensed in rounded masses. The ground substance of the entire nucleus is profoundly disturbed, and all of the basophilic chromatin eventually margi-

nates on the nuclear membrane, except in the case of the salivary gland inclusions in moles, which are more basophilic than acidophilic. After fixation the material of which the inclusions are constructed is not easily removed by acetic acid, alcohol, chloroform and other solvents. It contains little or no masked iron or thymonucleic acid.[4] Incineration shows that the yellow fever inclusions are devoid of mineral matter.[5] L. E. and E. J. Rector[6] have found that the same observation holds for mature herpetic inclusions. In interesting contrast, a large amount of mineral, especially calcium, occurs in the nucleoli and basophilic chromatin.

Type *B* inclusions occur in:

1. Poliomyelitis
2. Borna disease (from I. A. Galloway)
3. Kidneys of sewer rats (from E. Hindle)
4. Livers of mice (from G. M. Findlay)
5. Rift Valley fever (from J. R. Hudson)
6. Gliomas (from D. Russell)
7. Many species unaccompanied by evidence of disease

The reaction is localized in certain areas of the nucleus, where acidophilic droplets make their appearance. These often look hyaline and may be of small or large size. The nucleoplasm in which the inclusions are embedded may not be noticeably altered. Basophilic chromatin fails to marginate on the nuclear membrane. It may even accumulate to some extent on the centrally placed inclusions. The process seldom goes on to complete nuclear degeneration, and it is not accompanied by the marked reaction of tissue frequently but not always present with the type *A* inclusions. Such inclusions can be distinguished from nucleoli by: (1) recognition in the same nucleus of nucleoli stained differently; (2) their range of variation in number and size; (3) absence in them of detectable amounts of mineral and so on. It is unsafe to assume that different *B* inclusions are of similar composition. Their analysis has not been energetically pushed.

Are inclusions of type A and B distinct or simply different expressions of the same process?

That the latter may be the case is shown by preparations of rabbit encephalitis kindly submitted by Dr. C. C. Levaditi, which are exceptional in that they contain both. The material available seems to indicate that the type *B* inclusions represent less drastic modifications which may develop slowly and do not necessarily end in nuclear distintegration.

4. Cowdry, E. V.: Science **68**:40, 1928.
5. Cowdry, E. V.: Am. J. Path. **9**:149, 1933.
6. Rector, L. E., and Rector, E. J.: Am. J. Path. **9**:587, 1933.

In the animal series as a whole they are of much more frequent occurrence than the type *A* inclusions. Evidence that they are related to a virus, though satisfactory in some cases, is often lacking.

How far do variations in histologic technic modify the appearance of the inclusions?

No special technic is required for the identification of nuclear inclusions. They may be seen in sections of tissues fixed in formaldehyde solution or in Zenker's fluid and stained with hematoxylin and eosin or with eosin and methylene blue, indeed by any method which brings to light the so-called acidophilic and basophilic materials. Recognition depends on easily detectable morpholgic alterations in the nucleus as much as it does on changes in staining reaction. Only the inclusions caused by the virus of yellow fever have been investigated in detail in the fresh state, though several of the others have been seen. Fixation does not much modify either the morphologic character or the arrangement of the inclusions. But some fixatives, like Zenker's fluid, produce an additional coagulation of the nucleoplasm not seen after the use of fixatives rich in osmic acid.[7] Obviously preservatives leading to a swelling or a shrinkage of the nuclei alter the apparent width of the clear halo about the inclusions. Quite noticeable is the influence of the fixative on the staining reaction of the inclusions. Ordinarily the inclusions are acidophilic. Following treatment with Carnoy's fluid (absolute alcohol 6; chloroform, 3; acetic acid, 1) they are often distinctly basophilic.[8] When the color contrast is sharp, the inclusion material is generally less intensely stained with acid dyes than the acidophilic nucleolus or plasmosome.

Is there a sharp line of distinction between the formation of inclusions and nuclear changes unrelated to viruses?

Acidophilic material normally occurs in a great many nuclei, especially in those of nerve cells. In ordinary oxychromatic degeneration this increases in amount, though the mode of increase seems to differ, in a manner hard to specify, from that involved in the development of type *A* inclusions. A clear halo is usual about the mature type *A* inclusion which is not seen about the acidophilic material in cases of oxychromatic degeneration. This difference may result from the fact that when nuclei are first affected by this group of viruses they increase in size slightly or very considerably—an alteration not found in oxychromatic degeneration. In later stages of the reaction they usually shrink, and the halo becomes obliterated, so that the resemblance to oxychromatic degeneration is quite striking. While margination of chromatin also takes place in oxychromatic degeneration it does so at a later time,

7. Cowdry, E. V., and Kitchen, S. F.: Am. J. Hyg. **11**:227, 1930.
8. Cowdry, E. V.: Arch. Path. **10**:23, 1930.

as a rule after the nuclei have begun to shrink; it is rare to find all the central parts of large, robust or hypertrophied nuclei swept clear of basophilic chromatin in quite the same fashion as in these virus diseases. Though a sharp distinction cannot be made, and it appears that one is dealing with a process of nuclear disintegration peculiar in only minor respects when brought about directly or indirectly by viruses, there are, nevertheless, some marked differences. In the formation of type *A* inclusions the nuclei are always successively involved. Some look altogether normal, others show early stages in the reaction, and still others, late stages; whereas in oxychromatic degeneration most nuclei belonging to neighboring cells of the same sort exhibit exactly the same changes.

In autolytic postmortem nuclear degeneration some acidophilic material may be seen, depending on the kind of nucleus involved, and the basophilic chromatin eventually fades away without much margination. This change also spreads over nuclei of a given type almost uniformly, which again differs from the individuality in the formation of type *A* inclusions.

Since the nuclear alterations that accompany the formation of type *B* inclusions are less marked, there are fewer related properties to contrast. The acidophilic bodies that my collaborators and I have seen in the nuclei of hepatic cells of 22 per cent of dogs examined look, at first sight, like type *B* inclusions because the chromatin is not typically marginated, simply being pushed aside, and complete nuclear degeneration does not follow; but they are distinctly crystalline (see Brandts' figures [9]). In the epididymis the nuclei undergo remarkable alterations not so regularly evident anywhere else in the body, which have been interpreted by some as indicating the formation of a secretion product, and by others, as being degenerative products. The nuclei in the epididymis illustrated by Hammar,[10] Heidenhain and Werner,[11] Benoit [12] and Ludford [13] contain masses of material that very closely resemble type *B* inclusions. Another region where nuclear inclusions apparently occur constantly in man and certain species of animals, in the absence of disease, is in nerve cells of the nucleus supra-opticus and paraventricularis of the midbrain. Details and a review of the literature, which extends back many years, may be found in the papers of Scharrer and Gaupp [14] and of Scharrer.[15] The inclusions are interpreted by the authors

9. Brandts, C. E.: Beitr. z. path. Anat. u. z. allg. Path. **45**:457, 1909.
10. Hammar, J. H.: Arch. f. Anat. u. Entwcklngsgesch. (supp.) **1**:42, 1897.
11. Heidenhain, M., and Werner, F.: Arch. f. Anat. **72**:556, 1924 (fig. 9).
12. Benoit, M. J.: Arch. d'anat., d'hist. et d'embryol. **5**:175, 1926 (fig. 80).
13. Ludford, R. J.: Proc. Roy. Soc., London, s. B **98**:353, 1925 (fig. 11).
14. Scharrer, E., and Gaupp, R.: Ztschr. f. d. ges. Neurol. u. Psychiat. **148**: 766, 1933 (fig. 2).
15. Scharrer, E.: Frankfurt. Ztschr. f. Path. **27**:135, 1934 (fig. 5).

as intranuclear stages in the formation of a secretory product, and the claim has been advanced that the area is to be regarded as constituting an additional endocrine organ, the *Zwischenhirndrüse*. The bodies in the epithelium of the stomach figured by Carlier [16] resemble Borna inclusions. If such inclusions were found regularly in a virus disease and were absent in normal controls they would certainly be classified as type *B* inclusions.

Do all nuclear inclusions indicate virus action?

Types *A* and *B* are of a different status. It was with reference to inclusions like those in the salivary glands of guinea-pigs and in herpes, which belong to type *A*, that Cole and Kuttner [17] expressed the view that when typical inclusions are found, the presence of a filtrable virus is to be assumed unless its absence can be proved experimentally. This is going too far, but one naturally looks for a virus when such inclusions are observed. Often a virus has been discovered, but there are exceptions. These failures will be interpreted differently depending on experience and point of view. Some will say that the conditions were not favorable for the demonstration of virus action, while others will entertain the possibility that some type *A* inclusions may be produced in the absence of a transmissible virus. The question of how this may conceivably be done will be considered later.

With inclusions of type *B* the probability of a virus etiology is rather less. While a few of them appear regularly in cells injured by certain viruses, the vast majority are not known to be associated with viruses. Wolf and Orton [18] observed nuclear inclusions resembling those in poliomyelitis in many other diseases of the nervous system. Such inclusions seem to crop up without rhyme or reason, as shown by the same authors' studies on brain tumors [19] and by our own survey of the tissues of wild animals. Consequently with these type *B* inclusions the existence of a virus should not be taken for granted. They may be simply the expression of nuclear modifications occurring not only in some virus diseases but also in many conditions for which viruses are probably not responsible.

Does the same virus produce similar inclusions in cells of different types?

As a rule viruses are rather selective in their action. The virus of herpes is one of the most "cosmopolitan." The inclusions which it produces in a wide variety of cells are similar and of type *A*. When the submaxillary virus of guinea-pigs is injected intracerebrally it leads

16. Carlier, E. W.: Cellule **16**:405, 1900 (plate III).
17. Cole, R., and Kuttner, A. G.: J. Exper. Med. **44**:855, 1926.
18. Wolf, A., and Orton, S. T.: Bull. Neurol. Inst., New York **2**:194, 1932.
19. Wolf, A., and Orton, S. T.: Bull. Neurol. Inst., New York **3**:113, 1933.

to the formation of inclusions true to the original type *A* in the salivary glands, but without the extraordinary hypertrophy and the accompanying cytoplasmic inclusions which are marked features of the salivary gland reaction. No records are available as to the action of the viruses that produce type *B* inclusions on cells different from those primarily affected. The factor leading to their development is more local and does not exhibit the same spread to other tissues. As far as an answer is possible it is, therefore, in the affirmative.

How dissimilar are inclusions produced by different viruses in the same cell types?

If one excludes all other manifestations of virus action, are the inclusions of themselves pathognomonic? A definite answer is available only for those viruses which can be induced to act not simply on the same kinds of cells but also on individuals of the same species. This requirement has been fulfilled in only two instances. Herpes virus and virus III produce in the testicle of the rabbit inclusions of type *A* which are slightly different in degree of basophilia, while the herpetic virus and the virus of yellow fever, acting on the hepatic cells of the monkey (Cebus), cause the development of inclusions of different morphology. If identity of species is not insisted on, the material on which this paper is based permits a comparison of inclusions caused by a number of viruses in nerve cells, renal cells and hepatic cells; but the comparison is not so close as in the rabbit testicle, for the respective tissues were not fixed in the same bottle, embedded in the same block of paraffin and the sections mounted and stained on the same slide.

In nerve cells the inclusions produced by the Borna virus in horses, the virus of mad itch in dogs, the virus of louping ill in mice and the virus of poliomyelitis in monkeys are quite different. But to distinguish those in herpes, mad itch, the rabbit encephalitis of Levaditi and in guinea-pigs' brains inoculated with the salivary gland virus, which are all of type *A*, would not be so easy. It has not been tried under controlled conditions. In renal cells the inclusions caused by the Lucké virus in frogs and the Hindle virus in rats are not at all the same. In hepatic cells the inclusions excited by the herpetic and yellow fever viruses (already alluded to) and by the virus of fox encephalitis are different in shape and arrangement. To distinguish, however, the inclusions in owls and in parrots and parrakeets from the herpetic or yellow fever ones may be difficult or impossible. On the other hand, the inclusions occurring without obvious explanation in dogs [20] are so similar to those in fox encephalitis [21] as to suggest that the same virus may be responsible.

20. Cowdry, E. V., and Scott, Gordon, H.: Arch. Path. **9**:1184, 1930.

21. Green, R. G.; Kalter, M. S.; Schillinger, J. E., and Hanson, K. B.: Am. J. Hyg. **18**:462, 1933.

What is the site of action of these viruses and what is their means of spread?

In these, as in so many other respects, viruses behave differently. That some of them pass through the cell membrane and enter the cytoplasm is likely, but whether they always do so is one of the many unanswered questions. In late stages of the disease reaction, when the concentration of virus is greatly increased, and more and more cells become involved, the injury may be primarily due to the liberation of toxic substances which may operate in many ways. It is possible that they go one step farther and enter the nuclei. The original contention of Goodpasture and Teague [22] that the inclusions are masses of virus is, however, giving way.

Mechanical injury frequently opens the path for cytoplasmic invasion. Goodpasture [23] furnished interesting evidence that the herpetic virus may enter the traumatized peripheral processes of nerve cells and travel all the way to the nerve cell bodies, not in the tissue spaces between the nerve fibers, but in the substance of the axons or dendrites within the myelin sheaths. The physical factors involved are difficult to comprehend. The processes are tubes of capillary size through which no chemical substance could travel without being arrested by practically complete adsorption on the walls. Moreover, the microdissections of de Renyi [24] show that the axonic substance is of gelatinous consistency. When the myelin sheath is removed, it retains its filamentous shape and can even be cut in segments, which likewise hold their form. It is not an instance of passage through a thin aqueous material of low viscosity. One is obliged to hypothecate a spread by local increase in amount of virus in a medium devoid of nuclei and consequently incapable of developing nuclear inclusions. Other examples of increase in virus in the absence of nuclear inclusions will be given later. Sufficiently delicate cytologic methods have not been applied to ascertain whether the extension by progressive formation of more virus is related to detectable alterations in the medium. Nor have attempts been made to discover whether there are concurrent changes in the functional activity of the nerve fibers by utilization of the cathode oscillographic technic employed by O'Leary, Heinbecker and Bishop [25] for the investigation of physiologic changes in nerve fibers in experimental poliomyelitis. This is a key problem that calls for careful analysis.

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22. Goodpasture, E. W., and Teague, O.: J. M. Research **44**:139, 1923.

23. Goodpasture, E. W.: Medicine **8**:223, 1929.

24. de Renyi, G.: Architecture of the Nerve Cell, in Cowdry, E. V.: Special Cytology: The Form and Functions of the Cell in Health and Disease, ed. 2, New York, Paul B. Hoeber, Inc. 1932, vol. 3, p. 1370.

25. O'Leary, J. L.; Heinbecker, P., and Bishop, G. H.: Arch. Neurol. & Psychiat. **28**:272, 1932.

There is evidence that the neurotropic viruses—herpes, poliomyelitis, Borna disease, perhaps others, and rabies, which does not fall in the scope of this paper because it does not produce nuclear inclusions—extend not only to the nervous system in this remarkable manner, but also spread from the nervous system, once they have established themselves there, in the substance of the nerve fibers in a peripheral direction. These particular viruses can be isolated with difficulty or not at all from the blood stream. Other viruses, which do not possess this affinity for nerve tissue, or for which nerve tissue is not selectively vulnerable (which is not exactly the same thing), choose the blood as the means of transport but in varying degrees. Some spread rapidly in it and in large amounts while others remain quite sharply localized in particular tissues or organs. These differences in mode of spread have not as yet been correlated with the physicochemical properties of the viruses. Even a beginning has not been made.

Can the attribute of inclusion formation be correlated with virus properties?

If size of particle is a measure of diffusibility, it should be feasible, when more data are available, to arrange the viruses in series and perhaps to associate this property with ability to produce inclusions. That the particles are electropositive or electronegative at a certain p_H also affords a chance of correlation with inclusion formation. Nothing has been done in these directions.

Some viruses are mutants. The change of smallpox virus to vaccinia virus with loss of ability to produce intranuclear inclusions was a mutation. If only more were known of the physical chemistry of these two a clue might be gained to the feature responsible for the formation of inclusions, present in the one and absent in the other. The yellow fever virus in monkeys is hepatotropic, forming inclusions in liver cells. Transfer to the brain of mice renders it neurotropic, with which change it loses the ability to produce inclusions in liver cells and acquires the property of doing so in nerve cells. Again an alteration in virus is definitely related to a change in inclusion production, but it has not been possible as yet to characterize the alteration.

The same virus may yield inclusions in some species and not in others, though it causes disease in both. Two instances may be cited. The virus of louping ill acts in several species in which intranuclear inclusions have not been reported, but they appear in mice.[26] The virus of mad itch produces nuclear inclusions in the rabbit, monkey and cow but not in the domestic pig [27] though the latter may die of the

26. Webster, L. T., and Fite, G. L.: Proc. Soc. Exper. Biol. & Med. **30**:656, 1933.

27. Hurst, E. W.: J. Exper. Med. **58**:415, 1933.

disease. This is important, for it shows that the attribute of inclusion formation can be suppressed by species differences without interfering with the pathogenicity or the increase in the amount of the virus.

The case of virus III is interesting. Rivers and Tillett [28] discovered that by passage of testicle emulsion in series through rabbits this virus made its presence known by an increase in virulence and the development of a marked capacity to produce inclusions. Evidently there was a progressive change in it with the acquisition of this ability, but again from the physicochemical standpoint there is ignorance as to the nature of the alteration.

Can cell properties be correlated with inclusion formation?

Species differences in cellular properties commonly control susceptibility to the action of viruses. The control may be second or third hand and dependent on the conditioning by the cells of the tissue fluid or blood plasma. Tremendous doses of a virus easily exciting inclusion formation in one species will fail utterly to produce inclusions or any sign of a specific reaction in a nearly related species. The cell type determines the reaction, but in a less rigid way than the species. Herpes naturally produces inclusions in epidermal cells; mad itch, in nerve cells, and yellow fever, in liver cells. In general, viruses prefer ectodermal and endodermal derivatives and neglect mesodermal ones, but mesodermal cells are not wholly immune. The virus of fox encephalitis is peculiar in its liking for endothelium. Blood cells, muscle, bone and cartilage cells seldom respond to viruses by the development of intranuclear inclusions.

It may or may not be significant that ectoderm and endoderm, either in the embryo or in the adult or in both, line surfaces communicating with the outside. Opportunity for virus action is a factor in the determination of inclusion formation. A potent herpetic virus, such as the H. F. strain, will call forth inclusions when placed in contact with a wide variety of cells in addition to epidermal ones. On the lip it does no particular harm, but in the brain it may kill. The salivary gland virus of guinea-pigs fails to produce a clinically recognizable condition in its home, the salivary glands. When injected into the brain of a young and susceptible guinea-pig it leads to the development of inclusions in nerve cells and macrophages and is often lethal.

No systematic attempt has been reported to relate the alacrity of the formation of inclusions by certain cells and the reluctance or refusal by others to differences in their nuclear structure. At first sight it might appear that those already possessed of more acidophilic nuclear material, like nerve cells, would be more prone to present inclusions.

28. Rivers, T. M., and Tillett, W. S.: J. Exper. Med. **40**:281, 1924.

At the opposite end of the series is the lymphocyte, which has a nucleus the richest of all in basophilic chromatin and which rarely exhibits inclusion formation.

Can cells be rendered more susceptible to virus action?

Only isolated experiments have been made with a view to facilitating or inhibiting the response by altering the physiologic state of the cells. Scott [29] found that ligation of gland ducts suppresses, and stimulation with pilocarpine promotes, the formation of nuclear inclusions in the submaxillary glands of guinea-pigs. Having in mind the influence of vitamin B deficiency on nerve cells and the frequency, already cited, with which these cells are affected by viruses, Lucas, Neff and I are trying to measure any difference that may exist in susceptibility to the herpetic virus between rats deficient in vitamin B and normal rats. Thus far the results have been unexpected. The resistance seems to be, not broken down, but slightly enhanced by the deficiency. Chor,[30] working with me, attempted without success to depress natural resistance to the poliomyelitis virus by splenectomy. When investigators do manage to alter the receiving tissue so that this virus "takes," or discover some susceptible animal other than the monkey, one may look for progress comparable with that in yellow fever, which resulted from Max Theiler's transmission of the virus to white mice.

What cellular changes are coincident with inclusion formation?

By sifting the inconsequential alterations from the essential ones, one may hope in the end to devise artificial means whereby the development of intranuclear inclusions can be brought about in the absence of virus. Marked hypertrophy of cytoplasm and nucleus is a feature of the influence of some viruses on certain cells. It is most evident in the salivary glands among viruses producing nuclear inclusions. A local osmotic factor is probably involved. When the same viruses act in the brain, hypertrophy is slight. With a number of other viruses it is hardly perceptible. Hyperplasia likewise depends on the particular modes of response of the cells involved. It is sometimes quite extensive in epidermal cells and altogether absent in nerve cells, which have sacrificed the power of multiplication at the altar of extreme specialization. The relation of speed of inclusion formation to the properties of the inclusions has not been analyzed, but is undoubtedly important. As stated, the common denominator in the formation of type *A* inclusions, which are the most typical of virus action, is a change that sweeps through the whole nucleus. It may be electrical, osmotic or both. Perhaps there is a determining or coincident alteration in intranuclear p_H.

29. Scott, Gordon H.: J. Exper. Med. **49**:229, 1929.

30. Chor, H.: Arch. Path. **15**:387, 1933.

The injection of indicators might not be feasible because the nuclei are so much smaller than those treated in this way by Chambers. The shifts in material under centrifugal force should be studied in different stages of inclusion formation.

Is it possible to produce inclusions by means other than viruses?

The first step in this direction was the observation of Akiyama [31] and of Heinbecker and O'Leary [32] that curious alterations simulating inclusion bodies appear in nerve cells after electrical stimulation. Davenport, Ranson and Terwilliger [33] found that similar changes could be produced by soaking nerve cells in hypertonic salt solutions. They suggested "that the nuclear inclusions observed pathologically may be the result of disturbed osmotic conditions in the cell."

Lee,[34] with the advice of Dr. J. L. O'Leary, carried the experiments much further. He injected strong dextrose solutions intravenously into cats and made preparations of the spinal ganglions at various times thereafter. He encountered the alterations mentioned in the preceding paragraph, unless the animals were allowed to survive more than three hours, in which event no change was seen, proving the temporary nature of this modification in the living animal. It is desirable to emphasize the fact that the change was visible in freshly isolated cells examined in isotonic mediums. In fixed and stained specimens the nuclear inclusions were radically different from those of type *B* and presented certain points of similarity and of dissimilarity to those of type *A*. They resembled the *A* inclusions in that there was a marked increase in acidophilic material, which became heaped up in the center of the nucleus and was separated from the nuclear membrane by a clear halo. They differed from the *A* inclusions (1) by a failure of basophilic chromatin to marginate on the nuclear membrane, (2) by the nucleolus in most instances retaining its central position and (3) by the fact that the process did not go on to complete nuclear degeneration. He then applied a powerful diuretic, salyrgan, both intramuscularly and intravenously, and produced intranuclear inclusions in the pancreas and other glands. Though the nuclei were not so drastically and immediately altered as by viruses, some of the inclusions produced (at from three to thirty days) were indistinguishable from those of type *A*. The nuclei exhibited central acidophilic material, a clear halo and margination of basophilic chromatin with destruction of nucleoli. These experiments are being continued.

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31. Akiyama, S.: Arb. a. d. Med. Univ. z. Okayama **1**:278, 1929.

32. Heinbecker, P., and O'Leary, J. L.: Anat. Rec. **45**:219, 1930.

33. Davenport, H. H.; Ranson, S. W., and Terwilliger, E. H.: Anat. Rec. **48**: 251, 1931.

34. Lee, J.: Proc. Soc. Exper. Biol. & Med. **31**:383, 1933.

Are the viruses which produce nuclear inclusions living organisms?

The belief that this is the case rests on at least four considerations:

1. Evidence that organisms have been seen in the inclusions. Goodpasture [35] emphasized the occasional basophilia of the inclusions and stated that this is often due to the coloration of small particles of microscopic size, which he has interpreted as elementary bodies—a stage in the life cycle of so-called Chlamydozoa. No proof is, however, forthcoming that the particles are anything other than tiny masses of nucleoprotein which are a little more acid (i. e., more basophilic) than the rest of the inclusion material. Pinkerton and Hass [36] observed that in certain tissue cultures the rickettsiae of Rocky Mountain spotted fever accumulate within nuclei and in their opinion resemble inclusion bodies, but Cowdry pointed out that the correspondence is questionable.

2. These diseases, like those due to bacteria, are transmissible in series, and in each affected individual there is an enormous increase in virus pointing, it is claimed, to multiplication. The strength of this argument in favor of an organismal nature is undeniable unless it should be possible to explain transmissibility and increase in virus on another equally plausible hypothesis.

3. As in an infection due to a living organism, virus diseases do not spring up de novo but always appear by extension from preexisting cases.

4. Time and again infectious diseases of long unknown etiology have ultimately been found to be caused by organisms of some sort. But there is no inevitability about it. We are here concerned with those viruses that cause the formation of nuclear inclusions, particularly of type *A*, not with viruses as a whole. Since none of the viruses thus far proved to be organismal (for example, those of heartwater of sheep [37] and psittacosis [38]) belong to this group the argument loses some weight, but must be borne in mind.

Are the viruses of this category inanimate substances?

Chemists are more ready to entertain this concept than bacteriologists. It is a question whether it is more difficult to think of hitherto unrecognized chemical processes or of living things different from those familiar to us.

1. The transmissibility in series and increase in virus during the course of the disease could perhaps be explained on the supposition.

35. Goodpasture, E. W.: Am. J. Path. **1**:1, 1925.

36. Pinkerton, H., and Hass, G. M.: J. Exper. Med. **56**:151, 1932.

37. Cowdry, E. V.: J. Exper. Med. **42**:231, 1925.

38. Lillie, R. D.: Pub. Health Rep. **45**:221, 1930.

purely as a working hypothesis, that the disease inducing viruses of this class are autocatalytic; in other words, that acting on cells they are capable of producing more of the substance of which they are composed.

2. The feature of spread of virus infection from previous cases is not altogether incompatible with the agent being nonliving. It is possible that the virus substance is developed on extremely rare occasions and, once formed, passes from animal to animal, given the opportunity of entry into a mechanically injured cell.

3. But the principal reasons in justification of the hypothesis that these viruses producing nuclear inclusions may be inanimate substances are: (*a*) They are particulate and much smaller than any known living things. (*b*) Some of them are not inactivated (or killed) by conditions lethal for living organisms. (*c*) In the process of chemical purification they do not behave like organisms. (*d*) The relatively pure product is unstable, like an enzyme. (*e*) None of them has as yet been cultured on artificial mediums without living cells.

4. To base contentions only on viruses that are transmissible in series and that produce easily recognizable disease is to view only a small corner of the picture. Cytologists are interested in the wide distribution of nuclear inclusions of type *A*. This far exceeds the occurrence of viruses the existence of which has been demonstrated by animal passage. It may be, as already mentioned, that failure to prove virus action means that the required experimental conditions have not been realized. But it is also possible that even in this restricted group of viruses one has to do with substances which are only rarely autocatalytic. On this theory the intranuclear inclusions which are observed so frequently in the total absence of signs of disease and which it is not feasible by present methods to create simply by transfer of tissue emulsion from animal to animal are produced not by a living agent or by an autocatalytic substance but as a result of special local conditions in the respective tissue which one may hope eventually to duplicate experimentally in the absence of a transmissible agent whether organismal or chemical.

What general conclusions can be reached?

One gains the impression of many data that are poorly correlated. But nuclear inclusions in virus diseases are definite cellular modifications. They can be regarded as the fingerprints of a special and limited group of viruses, which is small in comparison with that hodge-podge of agents to which the term "virus" is ordinarily applied. Moreover viruses, like human beings, may act without leaving fingerprints. The immediacy of relation between virus and inclusions is in question. The

viruses may not themselves produce the inclusions. They may merely initiate physicochemical changes that lead to their production in some cases and not in others. There is a chance that these alterations can be brought about without viruses. What the viruses are, no one knows. There is danger in assuming that they are all alike. Some may be living agents of a type already familiar, or of a type altogether different, while others may be inanimate chemical substances akin perhaps to enzymes.

Notes and News

University News, Promotions, Resignations, Appointments, etc.—Georges Dreyer, professor of pathology in Oxford University since 1907, died suddenly on Aug. 17, 1934, at the age of 61.

At the University of London, J. R. Marrack has been appointed to the university chair, London Hospital Medical College of Pathological Chemistry.

Carl O. Jensen of Copenhagen, famous on account of his success in transplanting carcinoma in mice in 1901, has died.

W. C. Hueper has been appointed pathologist in the newly founded Institute of Experimental Toxicology of the DuPont Company at Wilmington, Del.

Andrea Saccone and Alfred Aingrist have been promoted to associate professors of pathology in the New York Homeopathic Medical College and Flower Hospital, New York.

Philip B. Hadley, recently associate professor in bacteriology in the University of Michigan, and Arthur P. Locke, recently chief of biochemical research in St. Luke's Hospital, Chicago, have been appointed on the research staff of the Institute of Pathology of the Western Pennsylvania Hospital in Pittsburgh.

Jean Cantacuzène, professor of experimental pathology in the University of Bucharest for more than thirty years, has died. A memorial book in two volumes has been published in his honor by Masson et Cie, Paris.

Society News.—The second conference of the International Association for Geographic Pathology was held in Utrecht on July 26 to 28, 1934. The major topic was arteriosclerosis. One session was devoted to hepatic cirrhosis, the subject of the first conference at Geneva in 1931. The third conference will be held in 1937 at a place to be selected. The list of those present at the Utrecht conference contains one hundred and sixty-six names representing twenty countries; ten members of the committee for the United States were present.

Research in Bacterial Chemistry.—According to the *Journal of the American Medical Association*, the Medical Research Council in England will sponsor an investigation in bacterial chemistry. Financial provision has been made for an initial period of five years. The work will be carried on under the direction of Paul Fildes at the Middlesex Hospital in the Bland-Sutton Institute of Pathology and the adjoining Courtauld Institute of Biochemistry.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

HYPERACTIVATION OF THE NEUROHYPOPHYSIS AS THE BASIS OF ECLAMPSIA AND OTHER HYPERTENSIVE STATES. H. CUSHING, Am. J. Path. **10**:145, 1934.

From the observations presented the conclusions are drawn: (1) that the source of these hypertensive disorders (eclampsia, essential hypertension) lies in the posterior lobe of the pituitary body; (2) that the extent of basophilic invasion from the pars intermedia is a measure of posterior lobe activity, and (3) that excessive infiltration by these elements represents the histopathologic basis of eclampsia and essential hypertension in young persons and may possibly also be related etiologically to atherosclerosis of old age. Whether the general hypothesis herein advanced should or should not prove on further study to be in all its features wholly correct, it will nevertheless provide an incentive to include a detailed study of the neurohypophysis in forthcoming postmortem studies of disorders in which hypertension is a distinguishing feature.

FROM THE AUTHOR'S CONCLUSIONS.

CARDIOVASCULAR RENAL CHANGES ASSOCIATED WITH BASOPHIL ADENOMA OF THE ANTERIOR LOBE OF THE PITUITARY. H. E. MACMAHON, H. G. CLOSE AND G. HASS, Am. J. Path. **10**:177, 1934.

Two cases of basophil adenoma of the anterior lobe of the pituitary gland, one reported by Bishop and Close and the other by Cushing, have been discussed again from a cardiovascular renal standpoint. It is shown that the cardiovascular renal lesion which was present corresponds to the picture originally described as malignant nephrosclerosis by Fahr.

FROM THE AUTHORS' SUMMARY.

THE CYTOLOGICAL PICTURE OF AN INFLAMMATORY EXUDATE IN RELATION TO ITS HYDROGEN ION CONCENTRATION. V. MENKIN, Am. J. Path. **10**:193, 1934.

The observations reported suggest that the differential leukocyte formula in an area of acute inflammation is a function of the hydrogen ion concentration of the exudate. The cytologic picture seems to be conditioned by the p_H of the exudate surrounding the injured tissue. The present study indicates that the local acidosis which develops as the inflammatory reaction progresses can adequately account for the shift in infiltration from polymorphonuclear leukocytes to mononuclear phagocytes at the site of inflammation.

FROM THE AUTHOR'S SUMMARY AND CONCLUSIONS.

THE EFFECT OF PARATHYROID HORMONE ON THE CALCIFICATION OF THE DENTIN OF THE RAT INCISOR. I. SCHOUR, W. R. TWEEDY AND F. A. MCJUNKIN, Am. J. Path. **10**:321, 1934.

The principal changes were found in the calcification of the dentin. A primary hypocalcified stripe developed in the dentin that was being calcified immediately after the first injection, and a secondary hypercalcified stripe developed in the dentin that was calcified subsequently. The dentin reaction may depend on control by the parathyroid hormone of a fraction of the serum calcium.

SELECTION WITH THE MAGNET AND CULTIVATION OF RETICULO-ENDOTHELIAL CELLS (KUPFFER CELLS). P. ROUS AND J. W. BEARD, J. Exper. Med. **59**:577, 1934.

Methods and apparatus are described wherewith living Kupffer cells can be procured from the liver of the rabbit and the dog for study and cultivation in vitro. Almost none of these cells can be dislodged from the normal liver by forcible perfusion; but after they have taken up finely particulate matter (india ink, iron oxide) they come away in great numbers. When they have phagocytosed magnetic iron oxide they can be selected with a magnet from among the blood elements present in suspension with them; and they are obtainable in quantity by this means. They do poorly when plated in a thin plasma clot, failing to multiply or to assume their characteristic shape; but they flourish when allowed to attach themselves to strands of lens paper bathed in serum that is frequently changed. Bacterial infection of serum cultures of Kupffer cells from normal rabbits and dogs occurs only as the result of secondary contamination of the materials, whereas it regularly develops in cultures from animals with fever induced by the injection of nucleic acid or of killed Bacillus prodigiosus. Kupffer cells obtained under such conditions are abnormally active, and some can be washed out of the liver of sick animals in the absence of any preliminary phagocytosis of particulate matter. The facts have a bearing both on the conditions conducing to invasion of the blood and on the response of the Kupffer cells in the emergency. The characters of the isolated Kupffer cells and the results of tests of their presumptive functions will be described in later papers.

FROM THE AUTHORS' SUMMARY.

THE CHARACTERS OF KUPFFER CELLS LIVING IN VITRO. J. W. BEARD AND P. ROUS, J. Exper. Med. **59**:593, 1934.

The Kupffer cells procured from the liver of the rabbit and the dog for culture in vitro have the typical characters of clasmatocytes. They are readily discriminated from the monocytes of other organs. Their surface is extraordinarily sticky—far more so than that of blood leukocytes or of the clasmatocytes found in peritoneal exudates; and in consequence they are exceedingly difficult to handle in vitro. They put forth enormous, pellucid, circular membranes resembling those of the clasmatocytes of exudate but larger. Splenic clasmatocytes, on the other hand, put forth rather small, one-sided, ground-glass membranes like broad tongues. On comparing them with Kupffer cells and the clasmatocytes of exudate one perceives that they are not wholly identical in their characters, but have secondary peculiarities. However, there exist good morphologic reasons for grouping them together and terming them all reticulo-endothelial. Kupffer cells are notably sensitive to injury, surviving in Tyrode solution for a much shorter time than blood leukocytes. However, they can be readily cultured on lens paper in serum. Under such circumstances they scatter on the fibers and live separately, presenting the same general aspect as when in the liver; but in the course of proliferation they soon lose some of their pronounced characters, retaining such as are common to clasmatocytes in general. A considerable population of ordinary leukocytes exists in the hepatic sinuses over and above those circulating in the blood. During infection, their number may greatly increase. Several cubic centimeters of packed white cells have been obtained from the liver of a sick dog. The fact has been realized that leukocytes may stop a while in the liver, yet the extent of the accumulation which sometimes takes place seems deserving of stress.

FROM THE AUTHORS' SUMMARY.

THE EFFECT OF HISTAMINE ON THE HEALING OF EXPERIMENTAL GASTRIC DEFECTS. C. A. FLOOD AND E. L. HOWES, Surg., Gynec. & Obst. **58**:136, 1934.

The subcutaneous administration of histamine interfered with the healing of mucosal defects in the prepyloric portion of the stomach of the cat and the dog. It delayed but did not prevent healing. Histamine had little effect on the healing of a mucosal defect high on the greater curvature of the stomach. The amount of

histamine required to delay the healing of a prepyloric mucosal defect in the cat for two weeks was from 1 to 1.2 mg. per kilogram twice a day.

SUMMARY (W. C. HUNTER).

THE PATHOGENESIS OF HYDRONEPHROSIS. FRANK HINMAN, Surg., Gynec. & Obst. **58**:356, 1934.

The pathologic changes of hydronephrotic atrophy are types of the degeneration produced by pressure and anemia and are peculiar to the kidney. Complete obstruction of the excretory ducts of other glands produces almost immediate cessation of function and primary necrosis and atrophy. Primary renal atrophy following complete obstruction of a ureter is the exception and occurs only when there is an initial anuria.

Experimental modification of the secretory pressure does not influence the progressive changes in a related manner. Hydronephrotic atrophy shows the same ordinary rate of development both with water starvation and with forced diuresis. Lowering and raising the pressure by partial obstruction of the renal artery and vein hasten the rate of development, not through the influence of increased intrapelvic pressure, but because of the increased ischemia.

The degree of the structural repair and restoration of function which follow removal of the obstruction of the ureter varies not only with the extent of the injury which has been produced, which is proportional to the period of obstruction and to the freedom from infection, but also with the degree and manner of the functional stimulation. A gradually increasing excretory load, such as occurs with slow destruction of the opposite kidney, affects a more permanent and greater structural repair than a lesser stimulation, such as occurs when the opposite kidney has undergone compensatory hypertrophy, or a too sudden overload, as in the case of removal of the opposite kidney. An efficient compensatory mate diminishes and an insufficient kidney on the opposite side increases the potentiality of repair of hydronephrosis.

The pathologic changes of hydronephrotic atrophy have been found only in kidneys with internal glomeruli. The gross and microscopic changes occur most typically in the mammalian kidney, which has a hilus and a pelvis. However, this structural arrangement is not essential. Similar changes follow complete obstruction of the ureter or the primary excretory duct of the apelvic kidneys of reptiles, birds and amphibians. The microscopic changes which follow direct obstruction of the tubules in the pelvic type of kidney, such as occurs when a papilla has been tied, resemble those which follow indirect obstruction, such as occurs when the ureter has been tied.

The mechanical factor in the development of hydronephrotic atrophy is a backflow of urine into the venous system. In mammalian kidneys this backflow probably is at first pyelovenous and later tubulovenous. In the apelvic mesometanephroi of reptiles, birds and amphibians the backflow is tubulovenous. Whenever a backflow fails to occur on complete obstruction of the excretory duct, anuria develops, and the pathologic change of primary atrophy instead of hydronephrotic atrophy results.

AUTHOR'S SUMMARY (W. C. HUNTER).

DEMONSTRATION OF TRAUMATIC FAT EMBOLISM IN BLOOD AND FATAL AMOUNTS OF FAT. O. SUSANI, Arch. f. klin. Chir. **179**:463, 1934.

Susani states that fat embolism is caused by neutral fat, principally olein. For this reason, determinations of the total lipoids or of partial lipoids are of no diagnostic value. The normal neutral fat values as given in the literature are frequently grossly erroneous. He presents exact methods for determining the presence and the amount of neutral fat in the peripheral blood. In severe fractures considerable amounts of neutral fat may be demonstrated in the peripheral blood, frequently associated with shock. This increase is considered a manifestation of latent fat embolism. Alimentary hyperlipemia is a definite entity in which the

values of blood fat may far exceed the normal. The fatal amount of fat is dependent on the state of division of the fat. In a moderately coarse state of comminution smaller fractions of fat may be more fatal than they would be if they were in the crude state. Pure fat induces death from a cardiac failure, whereas emulsified fat is fatal because of asphyxiation.

EXPERIMENTAL GLOMERULONEPHRITIS. W. MASUGI, Beitr. z. path. Anat. u. z. allg. Path. **92**:429, 1934.

This work on rabbits was a continuation of previous work on glomerulonephritis in rats from the intravenous injection of antikidney serum. In the rabbit the clinical manifestations, the urinary findings, the retention of nitrogen in the blood and the structural changes of the kidney were identical with those considered characteristic of chronic diffuse glomerulonephritis in man. The reaction in the kidney is held to be an allergic one.

O. T. SCHULTZ.

EXPERIMENTAL HYPERERGIC CARDITIS AND ARTERITIS. E. JUNGHANS, Beitr. z. path. Anat. u. z. allg. Path. **92**:467, 1934.

Swine serum, which was used in Junghans' experiments, is toxic to the rabbit and evokes a local reaction at the point of first injection. Ten rabbits were sensitized to swine serum by from three to six subcutaneous injections of from 0.5 to 2 cc. each at intervals of from six to nineteen days. After sensitization the animals were given one or two intravenous injections of swine serum in amounts varying from 9.25 to 10 cc. Five of the animals received from three to five intravenous injections at varying intervals, with a maximum single dose of 20 cc. The animals were killed and the heart, aorta and pulmonary artery examined microscopically. The heart valves revealed fibrinoid swelling of the connective tissue and cellular proliferation; the walls of the coronary arteries were swollen and surrounded by granulomatous perivascular inflammation, and the muscle fibers were damaged. This series of reactions Junghans terms "hyperergic carditis." In the aorta and pulmonary artery the media was swollen, and all the coats contained areas of cellular infiltration. The vascular reaction is termed "hyperergic arteritis." It is identical with the arterial changes noted in rheumatic infection.

O. T. SCHULTZ.

WEATHER CHANGES AND APPROACHING DEATH. G. ORTMANN, Virchows Arch. f. path. Anat. **291**:237, 1933.

This is an investigation similar to that made by Struppler in Munich, which has been previously abstracted (ARCH. PATH. **15**:280, 1933). It is based on more than 16,000 deaths that came to necropsy in Berlin. As in Munich, there were noted periodic increases in the number of deaths. These fluctuations were correlated with changes in meteorological conditions. Such a study appears to have value only if the deaths submitted to necropsy in a large hospital represent all or an overwhelming proportion of the deaths that occur in the institution. Ortmann found that there was a distinct increase in deaths on those days when there occurred a change in weather due to the passage of a cold front or a warm front over Berlin.

O. T. SCHULTZ.

DEVELOPMENT OF COLLAGEN FIBRILS IN TISSUE CULTURE. L. DOLJANSKI AND F. ROULET, Virchows Arch. f. path. Anat. **291**:260, 1933.

The authors trace the development of fibrils with the staining reactions of collagen in the plasma medium of tissue cultures. A transformation of fibrin into collagen was observed, but the formation of collagen fibrils may and does occur independently of the fibrin network of the plasma coagulum. The fibrils stain by the Foot silver method. They are most numerous immediately about the cell

bodies, but were never observed within the cell body or in the elongated processes of the mesenchymal cell. Although they arise independently of the cell, their formation occurs under the influence of cellular activity. The cell exerts its influence on fibril formation in the plasma medium by means of a secreted material, possibly something in the nature of an enzyme. Low diffusibility of the secreted material is inferred from the greater degree of fibril formation immediately about the cells of the culture.

O. T. SCHULTZ.

RELATIONSHIPS OF THE THYMUS TO THE ENDOCRINE AND LYMPHATIC SYSTEMS. HANNA SCHULZE, Virchows Arch. f. path. Anat. **291**:461, 1933.

Injection of thyroxine into young animals, especially mice, led to atrophy of the thymus. The atrophy was due to a direct local destruction of lymphocytes and to increased emigration of lymphocytes from the thymus. In young guinea-pigs thymectomy regularly resulted in compensatory hypertrophy of the cervical lymph nodes and occasionally in hypertrophy of the spleen. The author interprets these findings as proof of a relationship of the thymus to both the endocrine and the lymphatic system.

O. T. SCHULTZ.

THE EFFECT OF HIGH TEMPERATURE ON THE ALIMENTARY TRACT AND KIDNEY. J. F. BRODSKY, Virchows Arch. f. path. Anat. **291**:589, 1933.

In this experimental study of occupational disease, sixteen dogs and three cats were subjected for variable periods to high environmental temperatures such as prevail in certain occupations. Diarrhea developed, as it does in workers in heated atmospheres. In the large intestine there were noted degeneration and desquamation of the superficial epithelium. Similar degenerative and desquamative changes were noted also in the pancreas and the kidney. In the more prolonged experiments fibrosis of the pancreas developed. Spivack had ascribed the diarrhea of workers in heated environments to excessive water drinking forced by thirst caused by loss of water from the body. The experimental animals did not consume unusual quantities of water. Brodsky ascribes the clinical manifestations and pathologic changes noted by him to intoxication. He recommends that workers in heated environments be subjected to examination every three to six months, especial attention being paid to the urine to detect renal damage.

O. T. SCHULTZ.

THE INFLUENCE OF PHOTODYNAMIC SUBSTANCES ON THE BLOOD LEUKOCYTIC PICTURE OF RABBITS. W. N. NEKLUDOW, Virchows Arch. f. path. Anat. **291**: 600, 1933.

Quinine hydrochloride or eosin in solutions of 0.1 and 0.01 per cent respectively was injected intravenously into rabbits and a study of the leukocytes of the peripheral blood made at varying intervals. In some experiments the solutions were subjected to ultraviolet irradiation before injection; in others the animals themselves were irradiated just before or just after the substance was injected. The solutions used caused temporary leukopenia followed by leukocytosis, with a shift to the left and an increase in pseudo-eosinophils. These changes were more marked if the solutions or the animals were irradiated.

O. T. SCHULTZ.

EXPERIMENTAL FETAL INFLAMMATION. F. WOHLWILL AND H. E. BOCK, Virchows Arch. f. path. Anat. **291**:864, 1933.

Previous study of human placentitis and fetal infection had established that the reaction of the fetal tissues is chiefly histiocytic. In this experimental study of fetal inflammation turpentine or suspensions of bacteria or of india ink were injected into the amniotic liquor of guinea-pigs or into the umbilical cord, skin,

muscles or internal organs of the fetuses. The injections were made at various stages of pregnancy. Fetal sepsis was most often bronchiogenic, as had been previously observed in the human material, and resulted from the aspiration of infected amniotic fluid. A morphologic reaction to bacterial invasion was not observed in the fetuses until near the end of gestation and was macrophagocytic. A histiocytic reaction to turpentine and india ink was observed early in pregnancy and was similar to that which occurs in coelenterates. Even after development of the circulatory system the reaction was primarily histiocytic. Late in gestation a few cells of myeloid origin appeared, but this phenomenon was always preceded by a histiocytic reaction.

O. T. SCHULTZ.

STUDIES ON [HUMAN] GONADOTROPIC HORMONES FROM THE HYPOPHYSIS AND CHORIONIC TISSUE WITH SPECIAL REFERENCE TO THEIR DIFFERENCES. C. HAMBURGER, Acta path. et microbiol. Scandinav., supp. 17, 1933, p. 1.

The studies reported have been limited to the effect on the ovary of gonadotropic hormones of human origin. Infantile mice and rats have been used for the tests. A main result of the work is that the stimulation of the ovary by hypophyseal hormone (urine of castrates) differs in type from that by hormones from chorionic tissue (urine of pregnant women).

Pathologic Anatomy

RUPTURE OF THE RIGHT AURICLE OF THE HEART. G. M. CLOWE, E. KELLERT AND L. W. GORHAM, Am. Heart J. **9**:324, 1934.

A case of apparently spontaneous rupture of the right auricle is reported in a supposedly normal person in whom trauma and excessive exertion were absent. Serial electrocardiograms showing changes suggestive of coronary occlusion are presented for the first time. Histologic study revealed a preexisting obliterating endarteritis with interstitial hemorrhage and infarction of the auricular wall, leading to rupture, a process essentially similar to that causing rupture of the ventricle. The clinical and pathologic features of rupture of the auricle based on a review of fifty-four cases in the literature, plus the one described here, are discussed. The large incidence of rupture of the auricle before the fortieth year of life, 47.7 per cent, as against 6.7 per cent for ventricular rupture, is pointed out.

AUTHORS' SUMMARY.

PARADOXICAL EMBOLISM OF THE CORONARY ARTERY. J. JACOBI AND OTHERS, Am. Heart J. **9**:414, 1934.

A case of acute coronary occlusion due to an embolus from an old thrombosis of the femoral vein in the presence of a patent foramen ovale is reported. There were no associated arterial or endocardial changes. The factors in its production are discussed briefly.

FROM THE AUTHORS' SUMMARY.

CORONARY ARTERIES IN RHEUMATIC FEVER. H. T. KARSNER AND F. BAYLESS, Am. Heart J. **9**:557, 1934.

Rheumatic fever regularly produces disease of the coronary arteries. Either inflammatory or fibrotic lesions or both are practically constant. Except for participation by Aschoff nodules, the lesions are not specific for rheumatic fever. Fibrinoid degeneration is suggestive but not diagnostic. Elastica degeneration appears to be especially severe. The coronary disease is irregularly distributed as to both the various divisions of the coronary tree and the individual members affected. Its relation to myocardial disease cannot be positively established, but the late myocardial fibrosis is greater than is to be expected from the early acute

myocarditis alone. The influence of the coronary disease on myocardial fibrosis is better explained by intimal fibrosis than otherwise. Rheumatic fever predisposes to fibrosis of the coronary arterial tree in early life and to what appears to be precocious coronary sclerosis; but, although this is probably a chronic inflammation, it has not been shown conclusively to be dependent on the acute degenerative and inflammatory lesions. The coronary arteries in rheumatic fever undergo a progressive sequence of inflammatory lesions which closely resemble those of the endocardium and pericardium. It is practically certain that severe myocardial damage is associated with the arterial disease. The resulting effect on myocardial efficiency appears to be of significance in the clinical management and prognosis of rheumatic heart disease.

FROM THE AUTHORS' CONCLUSIONS.

HODGKIN'S DISEASE OF THE LUNG. S. E. MOOLTEN, Am. J. Cancer **21**:253, 1934.

Study of the lesions in the lungs in eight cases of Hodgkin's disease leads Moolten to emphasize that such study concerns a primary inflammatory process of granulomatous nature rather than a neoplasm. The uniform infiltration of the structures suggests a diffusible virus or toxic substance. A "primary pleurogenous" form of Hodgkin's disease of the lung is described, apparently for the first time. In most cases a peribronchial interstitial pneumonia is the main lesion, associated with more or less extensive granulomatous parenchymatous pneumonia. In about 10 per cent of all cases of Hodgkin's disease in the lungs the primary lesion is in the lung.

THE RENAL LESIONS OF RHEUMATIC FEVER. J. L. BLAISDELL, Am. J. Path. **10**:287, 1934.

In a study of the kidney in sixteen cases of rheumatic fever, a perivascular inflammatory reaction of the acute nonsuppurative type, affecting the smaller arteries and arterioles, was present in eight cases. Evidence of perivascular scarring was noted in four cases, while a recurrent inflammation was met with in two. The inflammatory reaction is usually seen in the adventitia and periadventitial tissues, with occasional infiltration and destructive change in the medial coat. Intimal changes, consisting of an endothelial swelling and proliferation, are constant. Glomerular damage, which was well marked in only one case, is to be regarded as dependent chiefly on nutritional disturbances brought about by the vascular changes. Little evidence of active or healed inflammatory processes was met with in the glomeruli. No evidence of the specific vascular lesions described by Pappenheimer and Von Glahn was encountered in the cases studied. The lesions described, which in general bear a close resemblance to perivascular foci of inflammation found in the myocardium, may be looked on as constituting a definite type of interstitial nephritis. It is seldom, however, that sufficient alteration in structure occurs to justify a diagnosis of renal disease during life.

FROM THE AUTHOR'S SUMMARY AND CONCLUSIONS.

MICROGLIA-LIKE CELLS IN THE LIVER, SPLEEN AND KIDNEY. H. S. DUNNING AND L. STEVENSON, Am. J. Path. **10**:343, 1934.

By del Rio Hortega's original silver carbonate method of specific staining for microglia cells have been demonstrated in the liver, spleen and kidney of the rabbit which in morphology are identical with the nearly normal or very early transitional forms of microglia in the nervous system. In their reaction to injury and to intravital injections of trypan blue they have been shown to be identical with microglia. These cells have been demonstrated in a transitional stage with spiked processes like microglia and containing droplets of fat or granules of trypan blue. By the silver carbonate method of staining earlier transitional forms have been demonstrated that contain no visible amounts of fat or trypan blue. A more advanced

transitional form has been shown in preparations of the spleen of the rabbit to be a histiocyte or large mononuclear phagocyte without processes and containing droplets of fat, granules of trypan blue, blood pigment and engulfed lymphocytes.

FROM THE AUTHORS' SUMMARY.

POLYARTERITIS NODOSA. R. B. HAINING AND T. S. KIMBALL, Am. J. Path. **10**:349, 1934.

The inflammatory changes are not confined to the adventitia and periarterial tissue as indicated by the name "periarteritis nodosa." On the contrary all the vascular coats may be involved. The primary changes may take place in the media, resulting in aneurysm. Involvement of the intima may result in thrombosis. The name "polyarteritis nodosa" is suggested as a better term.

HISTOLOGICAL CHANGES IN THE CENTRAL NERVOUS SYSTEM FOLLOWING EQUINE ENCEPHALOMYELITIS. O. LARSELL, C. M. HARING AND K. F. MEYER, Am. J. Path. **10**:361, 1934.

The most constant feature is perivascular infiltration. There may be degeneration of the Nissl substance and necrosis of nerve cells. Cytoplasmic inclusions are found in nerve cells.

TRACHEO-ESOPHAGEAL FISTULA OF SYPHILITIC ORIGIN. C. J. BUCHER AND J. ONO, Am. J. Path. **10**:391, 1934.

A case of tracheo-esophageal fistula of syphilitic origin in a 42 year old Negress is reported. Other lesions were gummas of the liver and lymph nodes and incipient aneurysm of the aorta.

FROM THE AUTHORS' SUMMARY.

AMYLOIDOSIS IN TUBERCULOUS RABBITS. R. M. THOMAS, Am. J. Path. **10**:419, 1934.

Amyloid degeneration occurred in 52 per cent of 175 rabbits experimentally infected with bovine tubercle bacilli. The occurrence of amyloidosis was restricted to animals surviving longer than two months after infection. The frequency of occurrence was greatest after the eighth month (75 per cent). The organs affected were the spleen, liver and kidneys, the spleen being most frequently affected. There was a uniform tendency for the deposition of amyloid to occur in those animals that showed the most extensive caseation of their lesions.

FROM THE AUTHOR'S SUMMARY.

THE ADRENAL CORTEX IN MONGOLISM. L. C. HIRNING AND S. FARBER, Am. J. Path. **10**:435, 1934.

In mongolian idiots, as maturity advances, a definite hypoplasia of the suprarenal cortex becomes evident by the use of histologic methods in the measurement of the width of the permanent cortex of the suprarenal gland.

FROM THE AUTHORS' CONCLUSIONS.

SARCOID OF BOECK (BENIGN MILIARY LUPOID) AND TUBERCULIN ANERGY. MARION B. SULZBERGER, Am. Rev. Tuberc. **28**:734, 1933.

Sulzberger reviews the classification of cutaneous tuberculous manifestations into three more or less well defined groups, tuberculosis, the tuberculids and the sarcoid group suggested by Lewandowsky, Jadassohn and Martenstein. In a woman with tuberculous sarcoid of Boeck and typical sarcoid infiltration of the lungs there was a lasting hyposensitivity or anergy to tuberculin. Microscopically there were naked epithelioid cell tubercles without many lymphocytes and without

necrosis or caseation. No bacilli could be demonstrated. It is interesting to note that Boeck's sarcoid not infrequently affects other organs. Next to the pulmonary changes, the most frequent are the cystic changes in the phalangeal bones (absent in this case). Sometimes there are sarcoids of the spleen and liver and even a sarcoid-iritis.

H. J. Corper.

Generalized Thrombo-Angiitis Obliterans. W. Birnbaum, M. Prinzmetal and C. L. Connor, Arch. Int. Med. **53**:410, 1934.

An unusual case of generalized vascular disease is reported, probably a case of early thrombo-angiitis obliterans, in which autopsy was possible early in the course because of the involvement of vital structures (infarction in the suprarenal glands). Involvement of the cerebral, retinal, pulmonary, coronary, mesenteric, suprarenal, pancreatic, duodenal, hepatic, renal and prostatic vessels and of the vessels of the extremities was found. The possibility that thrombo-angiitis obliterans is more frequently a generalized disease is pointed out.

Authors' Summary.

Structural Changes in the Brain from Trauma. N. W. Winkelman and John L. Eckel, Arch. Neurol. & Psychiat. **31**:956, 1934.

Injuries to the brain cause a number of gross and microscopic cerebral lesions depending on the extent and the degree of the trauma. The authors attempt to describe the central nerve changes in seven cases which they also studied clinically. The most outstanding changes were subarachnoid bleeding with reactive pial phenomena which may ultimately result in pial-cortical adhesions; intracerebral hemorrhages with "maceration" of the brain tissue; occasional edema which, when present, causes general ischemia with ganglion cell changes, and generalized gliosis which tends to "disappear gradually." The foregoing changes among others less common suffice to explain the neurologic and mental pictures in severe cases. According to the authors, changes in the brain are also the underlying cause of so-called traumatic neuroses.

George B. Hassin.

Splenitis Due to Uremia. K. von Wolff, Beitr. z. path. Anat. u. z. allg. Path. **92**:230, 1933.

Von Wolff describes changes in the spleen in uremia which he considers inflammatory. He divides his material into four groups: (1) cases with an increased number of leukocytes and proliferated pulp cells in the spleen; (2) those with serous, hemorrhagic and occasionally fibrinous exudate; (3) those with reticulo-endothelial proliferation, and (4) those with necrosis and necrobiosis. These various reactions are ascribed to the toxic action of protein degradation products on the spleen.

O. T. Schultz.

Changes in the Extrinsic Eye Muscles in Thyrotoxicosis. E. von Zalka, Beitr. z. path. Anat. u. z. allg. Path. **92**:239, 1933.

The external eye muscles of sixteen persons who died of exophthalmic goiter were examined histologically. Severe changes were noted. These included proliferation of sarcoplasm (Durante's cellular regression), lymphocytic infiltration and lipomatosis. Proliferation of sarcoplasm was seen in other conditions, but was less marked than in thyrotoxicosis. Lymphocytic infiltration is secondary to degenerative changes in the muscle and is not part of the lymphatism so frequently seen in exophthalmic goiter. The alterations described are held to be the result of the increased functional activity of the disease. They develop as the result of exophthalmos but may be a factor in bringing about an increasing degree of this condition.

O. T. Schultz.

PITUITARY BASOPHILISM. E. RUTISHAUSER, Deutsches Arch. f. klin. Med. **175**:640, 1933.

In three cases of osteoporotic obesity there was an increase in the basophil cells of the anterior lobe of the pituitary gland.

CONGENITAL SYPHILITIC KIDNEY WITH CARDIAC HYPERTROPHY AND CEREBRAL HEMORRHAGE IN AN EIGHT YEAR OLD CHILD. H. LUDTKE, Frankfurt. Ztschr. f. Path. **44**:405, 1933.

A child, aged 8, died from cerebral hemorrhage. The genesis of the contracted kidneys is traced to a syphilitic renal endarteritis. While the cardiac hypertrophy must be attributed to the contracted kidneys, the cerebral hemorrhage is considered due to both the high blood pressure and the syphilitic changes of cerebral blood vessels.

WILLIAM SAPHIR.

FATAL PULMONIC CIRRHOSIS PRODUCED BY MENTHOL. B. FISCHER-WASELS, Frankfurt. Ztschr. f. Path. **44**:412, 1933.

The autopsy on an 86 year old woman who gave the clinical picture of a progressively malignant pulmonic tumor revealed a massive tumor-like fibrosis involving regions about the hilus in both lungs. Histologic examination showed a dense scarlike connective tissue formation with numerous fat droplets. By histochemical examination these fat droplets were determined to be paraffin oil. Subsequently it was learned that the patient had suffered from a chronic nasopharyngitis and had used for more than twenty years, a 1 : 1,000 solution of menthol as nose drops. It must be assumed that the anesthetizing property of menthol and the chronic bronchitis favored the entrance of the oil into the bronchial tubes and alveoli. The presence of the oil in the tissue, acting on them as a foreign body, finally gave rise to the formation of the marked fibrosis.

WILLIAM SAPHIR.

CONGENITAL EPIDERMOID CYST OF THE HEART. A. DE CHATEL, Frankfurt. Ztschr. f. Path. **44**:426, 1933.

In a new-born girl, a cyst of the interauricular septum was found, which on histologic examination appeared to be a multilocular dermoid cyst containing derivatives of the three germinative layers. Chatel was unable to find record of a similar case in the literature. He is inclined to explain the dermoid cyst on the basis of misplaced ectodermal cells or on that of a metaplasia of entodermal cells in a very early stage of development. A meningo-encephalocele and a defect of the nasal septum were also found at autopsy.

WILLIAM SAPHIR.

TWO RARE HEMORRHAGES INTO THE ESOPHAGUS AND STOMACH. S. SCHEIDEGGER, Frankfurt. Ztschr. f. Path. **44**:527, 1933.

In one instance the hemorrhage was due to ruptured esophageal varicose veins the condition of which was attributed to an old thrombosis of the portal vein. It seems most probable that the thrombosis was the result of a severe appendicitis which occurred in childhood. In the other instance the fatal hematemesis was due to a congenital anomaly of submucous gastric arteries, which were very wide and tortuous. Following a meal one superficial branch became damaged and ruptured, with the subsequent fatal hemorrhage. In both patients the changes apparently had been present for many years but had not given rise to clinical symptoms.

WILLIAM SAPHIR.

BLIND ARTERIES WITH INVAGINATION OF THE INNER COATS IN THE REGION OF THE DUCTUS ARTERIOSUS. E. FRITZ, Frankfurt. Ztschr. f. Path. **45**:273, 1933.

In a new-born infant who had lived four and one-half hours a small blind artery was found in the loose connective tissue between the ductus arteriosus and the left bronchus. Close to the distal end of the artery its lumen revealed inverted portions of intima and media, which in some sections appeared free within the lumen, seemingly not attached to the wall. Another artery, also with inversion of its intima and parts of the media, was found between the ductus arteriosus and the left bronchus. The walls of the vessel revealed no pathologic changes. The belief is expressed that the inversion of the inner coats might have been the result of trauma which, however, could not be demonstrated. The absence of hemorrhage or any inflammatory changes speaks against birth trauma as the etiologic factor.

O. SAPHIR.

ECHINOCOCCUS DISEASE IN THE HEPATIC DUCTS. W. S. SKLJANIK, Frankfurt. Ztschr. f. Path. **45**:278, 1933.

Two instances are described. In both the exact diagnosis was made at autopsy. Clinically, cholecystitis and cholelithiasis with obstruction of the hepatic ducts were diagnosed in one instance, and possible pancreatic tumor with subdiaphragmatic abscess, in the other. Both patients were markedly jaundiced. In both instances the involvement of the hepatic ducts by the echinococci was secondary, the liver having been involved primarily. The author concludes that in instances of sudden obstruction of the bile ducts combined with diarrhea the feces should be examined for echinococci. He also believes that an intermittent appearance of a tumor in the region of the liver is pathognomonic of echinococci involving the hepatic ducts. In every disease of the hepatic ducts combined with sudden obstruction of the ducts, echinococcus disease should be considered.

O. SAPHIR.

VARICOSE VEINS IN THE SMALL AND LARGE INTESTINES CAUSING FATAL HEMORRHAGE. F. FENSTER, Frankfurt. Ztschr. f. Path. **45**:316, 1933.

At the autopsy on a 56 year old man who had bloody stools a varicosity was found in the submucous veins of the ileum and colon. One of the veins had ruptured and caused the fatal hemorrhage. The veins showed a partial loss of the media. The elastic fibers, particularly, were absent. A primary malformation of the elastic and muscular constituents of the walls of the veins seemed the best explanation for the formation of the varicosity.

O. SAPHIR.

TOXIC LESION OF THE BRAIN IN EARLY INFANCY. F. HOLLINGER, Frankfurt. Ztschr. f. Path. **45**:346, 1933.

The literature of so-called fetal encephalitis is discussed. This disease, with the exception of a syphilitic meningo-encephalitis, is rare. An intra-uterine infection plays apparently no rôle in its causation. Five cases are described. In three the Wassermann test of the infants and mothers, respectively, was positive. The encephalitis was attributed to the therapeutic use of sulpharsphenamine. In one infant microgyria and porencephalia were thought to be the result of the encephalitis. In the other two infants the encephalitis seemed to be the result of a gastro-intestinal intoxication.

O. SAPHIR.

RHEUMATIC PERIVASCULAR SCARS OF THE MYOCARDIUM. F. WILD, Virchows Arch. f. path. Anat. **290**:116, 1933.

The fourteenth in the series of contributions by Klinge and his associates on the pathology of rheumatic infection is based on microscopic examination of the

139 hearts studied in the preceding communication (abstr., ARCH. PATH. **17**:113, 1934), to determine the relation of arteriosclerosis to rheumatism. The same classification of the cases, based on clinical and gross anatomic data, is used in this article. The lesion with which Wild deals is the end-stage of the rheumatic granuloma or Aschoff body; he considers it histologically characteristic of antecedent rheumatic infection. It is an oval or spindle-shaped lesion, sometimes just barely visible to the naked eye, situated about the smaller arteries of the myocardium. It consists of dense, often hyaline, fibrous tissue, which may be arranged about the vessel like the layers of an onion. It is the result of characteristic damage to connective tissue during the acute stage of the infection and is not a replacement fibrosis of primarily damaged muscular fibers. This lesion, Wild believes, may readily be distinguished from senile myocardial fibrosis, which is also perivascular, and from the interstitial fibrosis of coronary sclerosis, syphilis and other diseases. Wild's findings were as follows, the first figure being the number of cases in the group and the figure in parentheses the percentage with rheumatic scars: active rheumatic infection at the time of death, 5 (100 per cent); known previous rheumatic infection with endocarditis, 8 (87.5 per cent); probable rheumatic infection with endocarditis, 7 (71.4 per cent); endocarditis without clinical history or gross evidence of rheumatism, 20 (45 per cent); syphilis, 15 (40 per cent); chronic infection, 10 (40 per cent); arterial hypertension, 22 (22.7 per cent); acute infection, 13 (53.8 per cent); sepsis, 3 (33.3 per cent); chronic tuberculosis, 10 (40 per cent); miscellaneous, 26 (46.1 per cent). Such high percentages in diseases other than rheumatism might lead to the thought that the lesion which is held by Wild to be pathognomonic of rheumatic infection is not specific. Wild maintains that his figures are minimal ones and that they indicate the great frequency of rheumatic infection in and about Leipzig.

O. T. SCHULTZ.

ENCEPHALITIS ASSOCIATED WITH ARTERITIS. FRANCIS HARBITZ, Acta path. et microbiol. Scandinav., supp. 16, p. 101, 1933.

Attention is called to some rare forms of arteritis accompanied by confusing nervous reactions, the correct diagnosis being reached only after microscopic examination. A woman of 39 complained of double vision, strabismus, protrusion of the right eyeball and drowsiness. There was found: thrombophlebitis of the cavernous and superficial petrosal sinuses associated with encephalitis of the temporal lobe and arteritis. A 42 year old man who had suffered for ten years from periodic attacks of headache on the right side was found to have arteritis with secondary necrosis and inflammation in the right cerebral hemisphere. No bacteria were demonstrable in the inflamed vessels.

JACOB KLEIN.

AMYLOID DEGENERATION IN ANIMALS. A. HJÄRRE, Acta path. et microbiol. Scandinav., supp. 16, p. 132, 1933.

A review of amyloid degeneration in various animals is presented. The liver and spleen are particularly susceptible to amyloidosis in horses, birds and cats; the kidneys, in cattle and dogs. There is a severe form of amyloidosis of the skin and lymph glands in the horse. Generalized amyloidosis occurs in animals especially after chronic infections (in the horse after immunizing procedures in obtaining antitoxic serum).

JACOB KLEIN.

HEREDITARY DWARFISM IN THE MOUSE. TAGE KEMP, Acta path. et microbiol. Scandinav., supp. 16, p. 189, 1933.

Hereditary dwarfism in the mouse has been found in a strain brought from England. It behaves in inheritance as a recessive mendelian character depending on a single gene. Some of the endocrine glands in the dwarf are abnormal and

reduced in size—the thyroid gland, the suprarenal cortex, the gonads and the pituitary gland. The chief cause of the condition is considered to be a hereditary deficiency of the anterior lobe of the pituitary gland.

JACOB KLEIN.

TOTAL GANGRENE OF THE GASTRIC MUCOSA IN GRANULOPENIA. G. FALK and N. GELLERSTEDT, Acta path. et microbiol. Scandinav., supp. 16, p. 50, 1933.

In a woman, aged 50, with a diagnosis of aleukemic lymphadenosis, roentgen treatments were followed by a marked reduction of the granulocytes in the blood and death. The entire lining of the stomach was gangrenous.

RETICULO-ENDOTHELIOSIS. M. G. NORDENSON, Acta path. et microbiol. Scandinav., supp. 16, p. 255, 1933.

In a woman, aged 65, who died with severe anemia after an illness of about two and one-half months, there was found some enlargement of the liver, spleen and some of the lymph nodes; also, marked osteoporosis with red marrow in the femur and vertebrae. Microscopically there was a general proliferation of the reticulo-endothelial system in the liver, spleen, marrow and lymph nodes.

Pathologic Chemistry and Physics

BILE CHOLESTEROL. A. WRIGHT AND G. H. WHIPPLE, J. Exper. Med. **59**:411, 1934.

Under uniform conditions of diet the normal dog with a bile fistula will eliminate fairly constant amounts of cholesterol—about 0.5 to 1 mg. per kilogram of body weight every twenty-four hours. Diets rich in cholesterol (egg yolk) raise the output of cholesterol in the bile, but the increase is trivial (from 5 to 15 mg.) compared with the intake (1.5 Gm.). Calves' brains in the diet are inert. Bile salt alone will raise the cholesterol output in the bile as much and often more than a cholesterol-rich diet. Bile salt plus egg yolk plus whole bile gives the maximal output of biliary cholesterol—60 mg. each twenty-four hours. Hepatic injury (chloroform) decreases both the bile salt and the cholesterol in the bile. Blood destruction (hydrazine) fails to increase the cholesterol output in the bile, and this eliminates the red cell stroma as an important contributing factor. Certain cholagogues (isatin and sodium dehydrocholate) increase the bile flow but cause no change in the cholesterol elimination. The ratio of cholesterol to bile salt in the bile normally is about 1:100, but the bile salts are more labile in their fluctuations. The ratio is about reversed in the circulating blood plasma, where the cholesterol is high (from 150 to 300 mg. per hundred cubic centimeters) and the bile salt very low. In the bile cholesterol runs so closely parallel to bile salt that one may feel confident of a physical relationship. In addition there is a suspicion that the cholesterol of the bile is in some obscure fashion linked with the physiologic activity of the hepatic epithelium.

FROM THE AUTHORS' SUMMARY.

BLOOD PLASMA CHOLESTEROL. W. B. HAWKINS AND A. WRIGHT, J. Exper. Med. **59**:427, 1934.

Hypocholesteremia with dissociation of the normal ratio of esterified to total cholesterol is related to chronic hepatic injury caused by chloroform. Hypercholesteremia may develop after prolonged biliary obstruction. Such hypercholesteremia may be promptly reduced below normal by chloroform poisoning or by an infection of the bile duct. Acute injury of the liver following chloroform anesthesia may cause no change in blood plasma cholesterol. Absence of bile in the intestine with faulty absorption of fat does not cause hypocholesteremia with dissociation of the ester ratio. Inadequate consumption of food or short periods of

fasting may cause no change in blood plasma cholesterol. Liver cells injured by chloroform may subsequently become resistant to chloroform. After prolonged biliary obstruction the liver is apparently more sensitive to small doses of chloroform by mouth. Analysis of blood plasma cholesterol may have a clinical application in differentiation between simple obstructive and parenchymatous lesions of the liver.

FROM THE AUTHORS' SUMMARY.

THE EFFECT OF CARROT FEEDING ON THE SERUM PROTEIN CONCENTRATION OF THE RAT. A. L. BLOOMFIELD, J. Exper. Med. **59**:687, 1934.

It has been shown that rats subsisting on a diet of carrots remain in good condition for periods of as long as twenty-one weeks. There are, however, loss of weight, at first rapid, later more gradual, and a fall in the serum proteins. When this drop is extreme (4.5 Gm. or more per hundred cubic centimeters) ascites and hydrothorax are likely to develop. The response of the individual rats varies greatly, however, even when they are all maintained under similar conditions, so that loss of weight and drop in serum proteins occur much more rapidly in some animals than in others. In the interpretation of the experiments, the point at issue is whether carrots contain an agent which has a disturbing effect on the mechanism regulating the concentration of the serum proteins or whether the drop in serum proteins is a nonspecific effect of malnutrition. The fact that controls on a variety of low protein, ill-balanced, vitamin-deficient diets failed to have edema and suffered for the most part only a slight, if any, drop in serum proteins below the physiologic level suggests that carrots exercise a deleterious influence. Further analysis has shown, however, that a diet of dried carrot powder leads neither to hypoproteinemia nor to edema (ascites), whereas the forced addition of water by suspending the powder in agar reproduces all the effects of fresh carrots. Water, therefore, seems to be the crucial factor rather than some specific constituent of carrots. Incidentally it may be noted that the artificial carrot offers a simple and certain method of producing hypoproteinemia in an animal otherwise in good nutritive condition, and it is proposed to use this technic for the study of further aspects of the subject. Finally it is of interest to correlate the carrot hypoproteinemia with clinical malnutritional disorders. It appears that a combination of factors is necessary in both cases, namely, an intake inadequate in total number of calories, an ill-balanced diet, a defective protein ration and a large fluid intake. No one of these factors alone seems adequate to produce hypoproteinemia, at least with any constancy.

FROM THE AUTHOR'S DISCUSSION.

RELATION OF MICROLITHS TO BILIARY CONCRETIONS. G. LEMMEL AND W. BÜTTNER, Beitr. z. path. Anat. u. z. allg. Path. **92**:262, 1933.

Gallbladder bile containing microliths, obtained at necropsy and at operation, was studied microscopically to determine whether microliths enter into the formation of macroscopic concretions. Growth of microliths by accretion, sufficient to form macroscopic concretions, was not observed, but the union of two or more microliths in a uniting ground substance may lead to the formation of structures visible to the eye. Such masses may form the centers of pigment-calcium calculi.

O. T. SCHULTZ.

SPECTROGRAPHIC DETERMINATION OF COPPER IN TISSUES. W. GERLACH AND K. RUTHARDT, Beitr. z. path. Anat. u. z. allg. Path. **92**:347, 1933.

In this contribution to Gerlach's series of studies on the spectrographic elementary analysis of tissues, a method is given for the detection and quantitative estimation of copper. The advantages of the method, as compared with the electrolytic procedure, are that it may be used on either fresh or fixed tissues, that it is rapid, and that only from 0.3 to 0.6 Gm. of formaldehyde-fixed tissue is necessary for an analysis.

O. T. SCHULTZ.

PHYSICAL AND CHEMICAL CHANGES IN THE SERUM OF INFECTED ANIMALS. E. REMY, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:57, 1934.

In guinea-pigs with tuberculosis, the examination of various physical properties showed no essential deviations from the normal; in guinea-pigs with trypanosomiasis, a slight lowering of surface tension was found. Tuberculous guinea-pigs showed a marked hyperglycemia and lower values for copper in the blood serum. The serum of guinea-pigs with trypanosomiasis had an increased reducing ability when tested with dichlorphenolindophenol. Syphilitic rabbits showed rises in nonprotein nitrogen and in dextrose.

I. DAVIDSOHN.

THE SUGAR CONTENT OF CEREBROSPINAL FLUID IN MENINGITIS. A. FLAUM, Acta path. et microbiol. Scandinav., supp. 16, p. 77, 1933.

In cases of bacterial meningitis the percentage of sugar in the cerebrospinal fluid is decreased. In meningitis caused by a "virus" the sugar content is not reduced. The decomposition of the sugar is not due to the cells in the fluid. The sugar may be fermented by bacteria; the virus of poliomyelitis does not break up sugar in the fluid.

JACOB KLEIN.

PATHOGENESIS OF SUBCUTANEOUS ADIPONECROSIS (SCLERODERMIA NEONATORUM). STURE A. SIWE, Acta path. et microbiol. Scandinav., supp. 16, p. 438, 1933.

Areas of subcutaneous infiltration may occur in infants on the shoulders, back, buttocks, cheeks and thighs. They disappear spontaneously leaving behind an atrophic skin associated with cyst formation and calcium deposits. The crystals in the subcutaneous tissue are composed of neutral fats, cholesterol esters and palmitic acid. In animals similar changes may be induced by the injection of palmitin and its acids. In the new-born there is a comparatively high content of palmitic acids.

JACOB KLEIN.

Microbiology and Parasitology

ACTINOMYCOSIS OF TUBES AND OVARIES. V. H. CORNELL, Am. J. Path. **10**: 519, 1934.

Seventy-one published cases of actinomycosis of the internal female genitalia are listed. Forty-five of the patients died, eight were improved, in seven the outcome was doubtful, and only eleven were possibly cured. The case reported showed involvement of both tubes and ovaries. The patient was operated on and treated by potassium iodide, and is well four years after operation. A tabulation of some features of the published cases is presented.

FROM THE AUTHOR'S SUMMARY.

THE INFECTION OF FERRETS WITH THE VIRUS OF SWINE INFLUENZA. R. E. SHOPE, J. Exper. Med. **60**:49, 1934.

The experiments described confirm the earlier observation of Smith, Andrews and Laidlaw that the virus of swine influenza is pathogenic for ferrets when administered intranasally. A disease that is clinically more severe and pathologically more extensive than that described by Smith, Andrews and Laidlaw is obtained if, when the virus is inoculated, the ferrets are under ether anesthesia. This disease may terminate fatally. Ferrets infected in this way show at autopsy an edematous type of pneumonia of lobar distribution. When stored in 50 per cent glycerol at refrigerator temperature the virus maintains its pathogenicity for ferrets for as long as seventy-five days. After serial passage through sixteen ferrets the virus is still

capable of inducing swine influenza when mixed with a culture of Haemophilus influenzae-suis and administered intranasally. Passage through ferrets causes no apparent attenuation of the virus for swine. Serum from pigs recovered from swine influenza is capable of neutralizing the ferret-passed virus for either swine or ferrets. Likewise serum from recovered ferrets neutralizes the virus of swine influenza for either ferrets or swine.

FROM THE AUTHOR'S SUMMARY.

THE RESPIRATION MECHANISM OF PNEUMOCOCCUS. M. G. SEVAG and L. MAIWEG, J. Exper. Med. **60**:95, 1934.

A virulent pneumococcus on being transformed into its avirulent form consumes many times more oxygen than the parent organism; but this gain of activity is a temporary property. After a time the pneumococcus degenerates into a form which consumes very much less oxygen than either the virulent or the recently derived avirulent form. These phenomena should receive consideration in any comparative study of the metabolic functions and oxidation products of virulent and avirulent strains of pneumococci. The change that takes place in the structure of the enzyme responsible for the carbohydrate biosynthesis during the shift from the virulent to the avirulent form may be associated with the changes in the enzyme structure already demonstrated in connection with these metabolic studies.

FROM THE AUTHORS' CONCLUSIONS.

SPONTANEOUS CONJUNCTIVAL FOLLICULOSIS OF RABBITS. P. K. OLITSKY, J. T. SYVERTON and J. R. TYLER, J. Exper. Med. **60**:107, 1934.

Spontaneous conjunctival folliculosis is widespread among various species of rabbits. It exists in two forms: type 1, in which the lesions are localized and the disease is relatively inactive, and type 2, in which the follicles are closely distributed over the entire surface of the conjunctiva and the process is more active and characterized by extensive inflammatory reactions. One type can be converted into the other either by experimental methods or by natural processes. The disease can be transmitted from rabbit to rabbit by means of subconjunctival inoculation of suspensions of the affected tissues or by instillation of such material into the conjunctival sac, or even by mere contact of folliculotic animals with those having smooth conjunctivae. It is plain that the disease is an infection. The causal agent is not filtrable through Seitz disks that retain Serratia marcescens nor through Berkefeld V candles that permit the passage of this organism. Furthermore, the lesions of the spontaneous or of the experimental disease do not exhibit the cytotropic effects or the inclusion bodies suggestive of the action of an ultramicroscopic virus. On the other hand, the lesions are characterized by a persistent, progressive chronicity and show certain resemblances to the granulomas. The evidence suggests that the spontaneous conjunctival folliculosis of rabbits is due to a micro-organism—one having a low grade pathogenic action. In a paper shortly to be published, a bacterium capable of reproducing folliculosis in normal rabbits will be described.

FROM THE AUTHORS' CONCLUSIONS.

VARYING INFLUENCE OF TUBERCULOUS RABBIT PLASMA ON THE GROWTH OF FIBROBLASTS IN VITRO. H. F. SWIFT, J. K. MOEN and E. VAUBEL, J. Exper. Med. **60**:149, 1934.

Plasma obtained from tuberculous rabbits within three or four months following their inoculation with bovine tubercle bacilli exerted a growth-inhibitory influence on transplants of rabbit fibroblasts, while that obtained after the fourth month was growth-stimulating. It is suggested that the inhibitory factor was linked in part with lipoidemia, while the stimulating elements were associated with leukocytosis.

FROM THE AUTHORS' SUMMARY.

THE FATE OF B C G and ASSOCIATED CHANGES IN THE ORGANS OF RABBITS. M. B. LURIE, J. Exper. Med. **60**:163, 1934.

Under the conditions of experiments in which 1 mg. of B C G is introduced intravenously into rabbits, the B C G multiply in the body, but they are soon destroyed, completely in most organs, all but completely in the lymph nodes. The remaining B C G persist in the lymph nodes for a long time, causing no tuberculous changes, and acquiring no added virulence for rabbits. The B C G produce typically tuberculous changes, sometimes extensive, which resolve completely. They cause caseation, but no softening. Acquired local immunity in tracheobronchial, mesenteric and axillary lymph nodes and in the spleen is shown to be less effective for a time than that in other organs. The destruction of bacilli begins with the appearance of cutaneous sensitivity to tuberculin and is at its height with maximum sensitivity. The secondary acute inflammatory reactions in and about tuberculous foci, the caseation and the hypersensitivity to tuberculin develop synchronously. Caseation and sensitivity to tuberculin do not occur early in the course of the disease in response to considerable amounts of bacilli and of tuberculin, but later they are incited by smaller amounts. FROM THE AUTHOR'S CONCLUSIONS.

RESULTS OF IRRADIATING STAPHYLOCOCCUS AUREUS BACTERIOPHAGE WITH MONOCHROMATIC ULTRAVIOLET LIGHT. F. L. GATES, J. Exper. Med. **60**: 179, 1934.

The incident energies required to kill Staphylococcus aureus and to inactivate its homologous bacteriophage have been measured at the various wavelengths of the quartz mercury vapor arc between 238 and 302 millimicrons and found to run strictly parallel, the readings for the Staph. aureus phage being obtained at a uniformly higher level of energy. This difference in levels is of less significance than the striking similarity in the shapes of the energy curves, which indicate that in both instances the same organic structures are absorbing the radiations. The results are open to three interpretations. The most obvious is that the bacteriophage is a submicroscopic organism. Again, it is possible that the bacteriophage is a product of its own lytic action on the homologous bacterium and contains the essential structural units which in Staph. aureus also are destroyed by ultraviolet rays, causing the death of the organism. A third, more remote explanation is that the phage, of wholly unknown nature, is absorbed on Staph. aureus material in so intimate a bond that the alteration of this material by irradiation renders the phage incapable of further lytic activity. FROM THE AUTHOR'S SUMMARY.

THE FIBRINOLYTIC ACTIVITY OF HEMOLYTIC STREPTOCOCCI. R. L. GARNER and W. S. TILLETT, J. Exper. Med. **60**:239 and 255, 1934.

The active fibrinolytic principle in cultures of hemolytic streptococci can be isolated in stable form and partially purified by the following methods: (1) precipitation of the culture filtrate with 3 volumes of 95 per cent ethyl alcohol; (2) adsorption on especially prepared aluminum hydroxide according to Willstätter followed by elution with tenth-molar sodium phosphate buffer, p_H 7.3. Concentration can be accomplished best by vacuum dialysis of either the culture filtrates or the preparations obtained by adsorption and elution. The streptococcic fibrinolysin is characterized by the following properties: 1. It may resist heating to 100 C. for sixty minutes; variations in thermal resistance are described. 2. Partially purified preparations give positive reactions in tests for protein. Activity is rapidly and completely destroyed by trypsin or papain. The active principle is demonstrable in dissolved fibrin even after eighteen hours' incubation.

The fibrinolysin of hemolytic streptococci exerts no hydrolytic action on casein, gelatin or peptone. The action on solid human fibrin is characterized by a small and gradual increase in the aminonitrogen content of the solution. The specific and special enzymatic action of fibrinolysin is contrasted with the actions

of trypsin and streptococcic peptase. Solutions of human fibrinogen, after brief incubation with fibrinolysin, lose the capacity to form fibrin. Solutions of rabbit fibrinogen, on the other hand, retain the property of transformation into fibrin even after prolonged exposure to fibrinolysin. Qualitative tests with solutions resulting from the action of streptococcic fibrinolysin on human fibrin indicate that the end-product may be protein and that the degradation of the molecule is not great.

FROM THE AUTHORS' SUMMARIES.

PULMONARY INFECTION IN PNEUMOCONIOSIS. H. O. PROSKE and R. R. SAYERS, Pub. Health Rep. **49**:839, 1934.

In general, the silicotic lung is more susceptible to bacterial infection than the average lung. This is probably due to the irritation of the respiratory tissues by the inhaled dust particles which weakens the mucous membranes and renders them susceptible to infection. The toxic influence of certain inorganic dusts on the tissues may be a contributing factor. The relation of tuberculosis to pneumonoconiosis has been studied to a considerable extent, but comparatively little work has been done in connection with other infectious processes of the lung, e. g., pneumonia, pulmonary abscess, bronchiectasis and influenza. An investigation was made of these conditions, both bacteriologically and experimentally, with the view of obtaining a better understanding of the predisposition to, and the mechanism of, infection of the lung in certain dusty trades. Bronchiectasis, abscess of the lung and gangrene occur frequently in hard-rock miners. It has been definitely established that aerobic pathogenic bacteria and certain fungi are responsible for these conditions, but the high percentage of cases in which the anaerobic microbes of the mouth and throat have been reported suggests that they at least participate in the production of the diseases. The responsibility of fusospirochetal organisms for severe infections of the respiratory tract had been suspected as early as 1867. In the past few years more than 2,000 cases of fusospirochetal abscess of the lung have been reported in the United States. Accurate figures on bronchiectasis of the same origin are not available. The mode of infection of the lungs in persons not engaged in dusty trades is briefly discussed and compared with the possible mechanism of infection in those having pneumonoconiosis. The bacteriology of fusospirochetal disease of the lung is given in detail, and a practical technic for the study of the anaerobic flora of the upper respiratory tract is appended.

FROM THE AUTHORS' SUMMARY.

BACTERIOLOGY OF THE TUBERCULOUS PRIMARY COMPLEX AT VARIOUS AGES. H. E. ANDERS, Beitr. z. Klin. d. Tuberk. **81**:260, 1932.

The question of the length of time that viable tubercle bacilli persist in calcified foci in lymph nodes has great biologic and clinical importance. Bibliographic research reveals no general agreement on this point. Bacteriologic studies on 157 anatomically healed primary foci have been reported. Tubercle bacilli were cultured from the lymph node component 36 times. Most of the positive cultures were obtained from persons above the fifth decade of life, and the negative cultures from those below the fifth decade. Few bacteriologic studies have been reported for intestinal primary complexes. Iizulia studied 39 such foci histologically without finding any bacilli. Anders studied 32 isolated primary pulmonary complexes and found viable bacilli in 6, an incidence of 21.4 per cent. All of these were from persons in the fifth or sixth decade of life. The negative ones were from persons between 20 and 40 years of age. No bacilli could be cultured from 14 healed intestinal foci. The conclusions are: that viable bacilli probably persist longest in those infected relatively late in life; that the source of endogenous lymphoglandular reinfection is not invariably the lymph node component of the primary complex as previously thought, but also, probably, lymph nodes in the same drainage area which show no histologic tuberculosis but contain viable bacilli, and finally that intestinal primary foci are probably only rarely the source of such endogenous reinfection.

AARON EDWIN MARGULIS.

EXPERIMENTAL SYPHILIS OF THE CENTRAL NERVOUS SYSTEM. T. TANI and H. FUNADA, Zentralbl. f. Bakt. (Abt. 1) **125**:423, 1932.

Rabbits were inoculated intracerebrally with testicular emulsions of four strains of spirochetes, three obtained from condylomas and one from a patient with yaws. Each strain initiated infection, as shown by the development of a positive Wassermann reaction of the spinal fluid. Tani and Funada conclude that this speaks against the idea of neurotropic strains of spirochetes. Furthermore, fourteen rabbits into which spirochetes from rat-bite fever, or Trypanosoma gambiense, were injected intracerebrally yielded positive Wassermann reactions of the blood but negative reactions of the spinal fluid. The conclusion, therefore, is that the antigenic substance which engenders the Wassermann reagin comes from the treponema.

Rabbits which had been infected with syphilis either in the testicle or the brain several months previously, and whose somatic organs were resistant to reinfection, were inoculated intracerebrally with spirochetal testicular emulsions. The spinal fluid in these animals became Wassermann-positive. The authors take this to mean that the central nervous system of rabbits is less resistant to syphilitic reinfection than are the somatic organs, rather than that there is a neurotropism of spirochetal strains.

PAUL R. CANNON.

THE EFFECT OF PHYSIOLOGIC SOLUTION OF SODIUM CHLORIDE ON STAPHYLOCOCCUS AUREUS. A. LAURELL, Acta path. et microbiol. Scandinav. supp. 16, 1933, p. 204.

Washing staphylococci with physiologic solution of sodium chloride had two toxic effects: (1) a bactericidal action due to oligodynamic influences in distilled water and (2) a detoxicating action, attributed to the sodium chloride ion, whereby the staphylococci become nonpathogenic in animals. No difference in agglutination titer was observed between staphylococci detoxicated in salt solution and those treated with formaldehyde.

JACOB KLEIN.

RESORPTION OF BACTERIA FROM THE GASTRO-INTESTINAL TRACT. ARVID LINDEN, Acta path. et microbiol. Scandinav., supp. 16, 1933, p. 225.

Behring, Ehrlich and others demonstrated that in new-born animals proteins and antitoxins pass unchanged from the gastro-intestinal tract into the blood. There is considerable disagreement about the question of resorption of microorganisms from the gastro-intestinal tract. Calmette's peroral vaccination against tuberculosis has awakened interest in this question. Linden gave india ink, carmine, timothy bacilli, bovine tubercle bacilli and B C G to young mice and guinea-pigs. Microscopic examination indicated that resorption of these substances and bacteria occurred rarely. The results of the study are not in agreement with Calmette's assumption of a general impregnation of the lymphoid system by the ingested bacteria.

JACOB KLEIN.

Immunology

NEMATODE AND CARCINOMA IN HUMAN KIDNEY PELVIS. ALFRED PLAUT, Am. J. Cancer **20**:610, 1934.

A painful abdominal swelling which had been noticed a few weeks before admission proved on examination to be a large kidney with stones. At operation many cysts were found with firm tissue between them, suggestive of a neoplasm. Six weeks after nephrectomy the patient was operated on again on account of swelling and pain. The broken-down tissue which was removed at this operation proved to be squamous cell carcinoma. The carcinoma, which obviously started from the renal pelvis, was densely infiltrated with eosinophil cells. The remainder of the renal tissue was severely inflamed and widely atrophic.

At autopsy, metastatic squamous cell carcinoma was found in the liver and retroperitoneal tissue. Calcified masses in the kidney were suggestive of remnants of a worm. Continued examination revealed a characteristic but unusually small nematode egg. This egg cannot belong to any of the nematodes which have been described in the kidney so far. The occurrence of parasitism and tumor of the kidney together is regarded as extremely rare in human and animal pathology. It is possible that the presence of the worm was a factor in the development of the carcinoma.

The patient was Russian and had been a resident of New York City for twenty-one years.

AUTHOR'S SUMMARY.

ACQUIRED AND GENETIC IMMUNITY. J. W. GOWEN AND R. G. SCHOTT, Am. J. Hyg. **18**:674 and 688, 1933.

The data herein presented show that the ability to survive a given inoculation of the virus of pseudorabies is markedly influenced by the genetic constitution of the animal. Susceptibility shows some tendency to be dominant in the F_1 cross. Comparison of a line which was resistant to pseudorabies and another which was susceptible for their respective resistances to another disease, mouse typhoid, showed their reactions to the second disease completely reversed. The F_1 cross for the second disease now show susceptibility largely recessive. These facts lead to the conclusion that genetic constitution as it is related to resistance to these diseases is perhaps best regarded as a composite of several distinct genes, some favoring resistance or susceptibility to one environmental agent and some to another.

Double mating is suggested as a genetic technic for distinguishing between acquired and inherited immunity. This technic seems to favor the hypothesis that resistance to inoculated Salmonella aertrycke in a selected strain of mice is due to a concentration of genetic factors for resistance and not to a transfer of acquired passive or active immunity.

FROM THE AUTHORS' SUMMARIES.

A STUDY OF THE GROWTH IN AREA OF INTRACUTANEOUS TUBERCULIN REACTIONS. C. A. STEWART, Am. Rev. Tuberc. **28**:844, 1933.

The degree of specific allergy to tuberculin induced in a group of children by a primary tuberculous infection varies widely in different ones. The average degree of allergy in children who have primary tuberculosis exclusively is represented by an area of about 30.2 sq. cm. for 0.1 mg. of old tuberculin. The area of the cutaneous reaction to tuberculin is not directly proportional to the amount of tuberculin used. The time required to attain the maximum area is directly related to the degree of allergy, and the relative rapidity with which the impulse to area increment is expended is inversely related to the degree of allergy.

H. J. CORPER.

THE AGGLUTINATION OF HEMOLYTIC STREPTOCOCCI BY PLASMA AND FIBRINOGEN. W. S. TILLETT and R. L. GARNER, Bull. Johns Hopkins Hosp. **54**:145, 1934.

Plasma from normal persons and from patients is capable of agglutinating, in very high dilutions, certain strains of hemolytic streptococci. Fibrinogen, chemically isolated from plasma, also agglutinates the same strains of hemolytic streptococci and is considered to be chiefly responsible for the reactivity of the plasma. Only those strains of hemolytic streptococci that are agglutinated by serums from persons harboring acute infection are visibly affected by plasma or fibrinogen; killing the organisms by heat destroys their reactivity in plasma and fibrinogen, as well as in serum; cultures, killed with formaldehyde, remain susceptible to the effect of each of the three blood constituents. The reactive fraction in the serums of patients seems to be a protein closely related to fibrinogen.

FROM THE AUTHORS' CONCLUSIONS.

THE RELATION OF ALLERGY TO IMMUNITY IN TUBERULOSIS. H. ROTHSCHILD, J. S. FRIEDENWALD and C. BERNSTEIN, Bull. Johns Hopkins Hosp. **54**:232, 1934.

Complete desensitization to tubercle bacilli and to tuberculin can be achieved in tuberculous guinea-pigs by a prolonged and properly graded course of subcutaneous injections of Koch's old tuberculin. The desensitizing power of purified tuberculoprotein is less than that of Koch's old tuberculin in proportion to its lesser power to produce allergic reactions. Long-continued daily subcutaneous injections of massive doses of concentrated glycerin broth in some instances desensitizes tuberculous guinea-pigs to tuberculin. This nonspecific desensitization is not due to the glycerin contained in the broth. It is not known whether this nonspecific desensitization is free from the danger of perifocal reaction. Infection in desensitized immune animals does not introduce into the histologic picture of tuberculous lesions features that are novel to the pathology of human tuberculosis. In all these respects the reactions of the desensitized animals were equal to or superior to those of the nonsensitized controls. So far as inhibition of the spread of lesions from the site of infection to the viscera may be used as evidence of a local fixation of bacilli, the desensitized, nonallergic immune animals were able to resist the spread of infection as successfully as the allergic ones.

FROM THE AUTHORS' CONCLUSIONS.

A STUDY OF THE CHARACTER AND DEGREE OF PROTECTION AFFORDED BY THE IMMUNE STATE INDEPENDENTLY OF THE LEUCOCYTES. A. R. RICH and C. M. MCKEE, Bull. Johns Hopkins Hosp. **54**:277, 1934.

When the circulating leukocytes are sufficiently reduced by benzene none emigrate at a site of infection, and the character of the protection afforded by an immune state, independently of that conferred by the activity of the leukocytes, can be studied. Immunized animals were treated with benzene to remove their leukocytes, and were then infected intradermally with virulent pneumococci of type I. In these immunized leukopenic animals the immune antibody influenced profoundly both the character of the bacterial growth and movement and the course of the infection. The bacteria, as they proliferated, adhere to themselves and apparently to the tissues as well, and were thus held fixed at the site of inoculation for hours after nonimmune controls had died of septicemia. The immediate local immobilization of the bacteria was demonstrable even in the absence of microscopically detectable inflammatory exudate or thrombosis of lymphatics. The latter factors are, therefore, not primarily responsible for the immobilization of the bacteria in the immune body, though when they are finally established they undoubtedly serve to assist in inhibiting the spread of the bacteria. Since the phenomenon of immobilization occurs in passively as well as in actively immunized animals, it is the antibody content of the fluids of the immune body which is primarily responsible for this prevention of the prompt spread of the bacteria. However, in the absence of the leukocytes the growth of the immobilized bacteria proceeds uninterruptedly until great colonies and masses of pneumococci have been formed at the site of infection, after which the bacteria penetrate into the blood and lymph streams and the animal dies with septicemia even though its plasma is potent in passively protecting nonimmune animals possessing leukocytes. If only relatively few leukocytes appear at the site of infection (far fewer than the number which appear in the lesions of nonimmune controls which succumb to the infection) the bacteria, which are opsonized by the antibody, are rapidly ingested and destroyed, and the lesion is sterilized. In addition to its opsonizing power, the humoral antibody, therefore, performs the important protective function of preventing the immediate spread of the bacteria throughout the body, holding them fixed at the site where they lodge until the phagocytic leukocytes are able to reach the spot and destroy them. It is, furthermore, the humoral antibody which, with the cooperation of the intravascular phagocytic macrophages of the liver and spleen,

brings about the rapid segregation and destruction of bacteria which do penetrate into the blood stream, thus further inhibiting the development of septicemia and metastatic infection; but acquired immunity creates no condition of the fluids or fixed tissues which can prevent the progressive and overwhelming growth of the bacteria in the absence of the phagocytes. FROM THE AUTHORS' SUMMARY.

PERIVASCULAR REACTIONS FOLLOWING INJECTION OF STREPTOCOCCI INTO SENSITIZED RABBITS. C. H. HITCHCOCK and others, J. Exper. Med. **59**:283, 1934.

Intravenous injection of small doses of nonhemolytic streptococci into previously sensitized rabbits is usually followed by the appearance of perivascular cellular aggregates in the lungs and liver. The characteristic cell in these aggregates is moderately large, with a vesicular nucleus, prominent nucleoli, clumped chromatin and basophilic cytoplasm. In addition, the lesions contain small lymphocytes and granulocytes. This lesion is easily differentiated by architecture and cell content from normally occurring lymphoid aggregates and from spontaneous rabbit hepatic cirrhosis. This mononuclear response does not occur when the intravenous dose is large enough to cause death of the animal within twenty-four hours. In the spleen and lymph nodes the characteristic basophilic cells which normally occur in these organs are present in increased numbers. Following intravenous treatment alone or sensitization without intravenous treatment, the lesions occur much less frequently and, when present, are smaller and more sparsely found. As, in the present series of experiments, this lesion was not found in normal animals and infrequently in those treated by the intravenous route alone, it is suggested that the preliminary sensitization serves to enhance the animal's reactivity to the antigen. In this way a small dose of bacteria is capable of eliciting the cellular phenomenon, which in unsensitized animals appears only when larger doses of antigen are administered over longer periods of time. Too large a dose of antigen, however, results in shock and cell death rather than in proliferation.

FROM THE AUTHORS' SUMMARY AND CONCLUSIONS.

A SEROLOGICAL DIFFERENTIATION OF BOVINE HEMOLYTIC STREPTOCOCCI (GROUP B). R. C. LANCEFIELD, J. Exper. Med. **59**:441, 1934.

Hemolytic streptococci of group B (derived chiefly from cattle) have been further subdivided by use of the precipitin reaction into specific types. With three exceptions, the twenty-one strains of group B were differentiated into three specific types. Chemical analyses of the type-specific substances of group B strains of types I and II show that they are polysaccharides (S substances). This is in contrast to the fact that proteins (M substances) were previously shown to determine type specificity among strains of human origin (group A). The group-specific substance, C, serologically identical in all members of group B, was also identified as of polysaccharide nature. FROM THE AUTHOR'S SUMMARY.

LOSS OF HEMOLYSIN AND PIGMENT FORMATION WITHOUT CHANGE IN IMMUNOLOGICAL SPECIFICITY IN A STRAIN OF STREPTOCOCCUS HAEMOLYTICUS. R. C. LANCEFIELD, J. Exper. Med. **59**:459, 1934.

A variant arising in a culture of a hemolytic streptococcus was shown to have lost the properties of producing pigment and hemolyzing blood. Despite the loss of these two functions, it had in common with the strain from which it was derived certain other distinguishing biochemical characteristics, as follows: Both attained the same hydrogen ion concentration in dextrose broth; both hydrolyzed sodium hippurate, grew on bile agar, and fermented trehalose but not sorbitol; both failed to reduce methylthionine chloride U. S. P. (methylene blue) in milk cultures and were insusceptible to the action of streptococcic bacteriophage. In addition, the

virulence of the variant remained the same as that of the original culture. The antigenic and serologic specificity of the variant was identical with the group and type specificity of the original strain (group B, type I). These specificities were established by the use of immune serums prepared by immunization of rabbits with each form. The immunologic reactions employed were reciprocal agglutination, precipitation, agglutinin absorption, precipitin absorption and passive mouse protection.

FROM THE AUTHOR'S SUMMARY.

STUDIES ON TYPHUS FEVER. H. ZINSSER and M. R. CASTANEDA, J. Exper. Med. **59**:471, 1934.

Guinea-pigs infected with European typhus virus can be passively protected with the serum of a horse that has been treated with killed Mexican rickettsiae.

FROM THE AUTHORS' CONCLUSIONS.

ON THE USE OF ADSORBENTS IN IMMUNIZATIONS WITH HAPTENS. J. JACOBS, J. Exper. Med. **59**:479, 1934.

Experiments are described which confirm the observation of Gonzalez and Armangué that heterogenic extracts can be made antigenic by adsorption to inorganic materials. With fractions of the original extracts from which a part of the inactive material had been removed no such enhancement was observed, whereas with foreign protein an activation was still possible. Carbohydrate preparations behaved similarly in that purification, perhaps loss of protein, was accompanied by a distinct decrease in antigenicity after adsorption. The activity of a but slightly antigenic hetero-albumose preparation was markedly increased after adsorption to charcoal and alum. The most reasonable explanation for the effects observed by Gonzalez and Armangué, and Zozaya, seems to be that a preexisting antigenic capacity has been enhanced by the use of adsorbents. The experiments reported here support the view that these effects are influenced significantly by the presence of substances other than those of a specific nature.

FROM THE AUTHOR'S SUMMARY.

HETEROPHILE ANTIGEN COMMON TO AVIAN ERYTHROCYTES AND SOME STRAINS OF PASTEURELLA. L. BUCHBINDER, J. Immunol. **26**:215, 1934.

A new heterophile antigen present in the erythrocytes of birds and in some strains of the group of bacteria concerned in hemorrhagic septicemia is described. In contrast to its apparent chance appearance in strains of Pasteurella, it is present in many birds in an orderly fashion. The significance of this heterophile antigen in avian erythrocytes is discussed from the standpoint of the evolution of species. Additional strains of the hemorrhagic septicemia organism containing Forssman's heterophile antigen are described.

FROM THE AUTHOR'S SUMMARY.

PNEUMOCOCCUS LEUCOCIDIN. F. ORAM, J. Immunol. **26**:233, 1934.

A toxin has been produced from actively growing cultures of Pneumococcus which destroys leukocytes, as demonstrated by the Neisser and Wechberg method. This leukocidin has been demonstrated in aerobic and anaerobic cultures, from growths of both virulent and avirulent strains and from types I, II and III. It is believed that a peroxide which is formed in the aerobic cultures modifies the potency of the toxins through oxidation. The peroxide appears earlier and is of greater concentration in cultures of the virulent strains; this may account for the avirulent cultures apparently containing toxins of as great a potency as those of the virulent strains.

FROM THE AUTHOR'S SUMMARY.

EFFECT OF IMMUNE YELLOW FEVER SERUM IN MONKEYS. N. C. DAVIS, J. Immunol. **26**:361, 1934. PROTECTIVE PROPERTIES AGAINST YELLOW FEVER VIRUS IN THE SERA OF THE OFFSPRING OF IMMUNE RHESUS MONKEYS. M. HOSKINS, ibid., p. 391.

Immune serum from recovered animals, when injected at from twenty-four to forty-eight hours following inoculation with the virus of yellow fever, was capable of preventing the fever or ameliorating it in a significant proportion of experimental monkeys. After forty-eight hours the effect was less definite. In no instance did the serum prevent death when administration was delayed until the temperature of the monkey had reached 104 F. (Davis.)

Five baby monkeys born of mothers immune to yellow fever were all found to have in their serum protective properties against yellow fever. This was the case when they were still subsisting on their mother's milk. In two instances in which the baby monkeys had been separated from their mothers for three weeks, the serum of the offspring showed no evidence of protective properties. (Hoskins.)

FROM THE AUTHORS' SUMMARIES.

IMMUNOLOGIC STUDIES IN TYPHOID FEVER WITH RELAPSES. G. D. C. THOMPSON and E. E. ECKER, J. Infect. Dis. **54**:177, 1934.

A case of typhoid fever with two relapses is reported which is of particular interest because of a complete lack of agglutination and precipitation reactions in the course of the disease while positive complement fixation and bacteriolytic and marked opsonic powers were observed, possibly indicating the final mechanisms of recovery. These results also appear to indicate a plurality of antibodies, but it is to be emphasized that the antigen is complex and that the question of unity or plurality of antibodies can be solved only by the use of a single and pure antigen. The case is also of importance in that the serum failed to dissociate the organism and because no bacteriophage was isolated. The organisms isolated proved to be typical typhoid bacilli (culturally and serologically) and stimulated the production of the O type of agglutinins in the rabbit.

FROM THE AUTHORS' SUMMARY.

GRANULAR AND FLOCCULAR TYPES OF AGGLUTINATION WITH TYPHOID BACILLUS. R. GILBERT, M. COLEMAN and A. B. LAVIANO, J. Lab. & Clin. Med. **19**:225, 1933.

The employment of macroscopic tests with two suspensions of killed typhoid bacilli, one to demonstrate the floccular or species-specific, and the other, the granular or group-agglutinative properties, usually furnishes information of greater diagnostic significance than the microscopic test with a living culture does. Apparently, agglutination in a 1:80 or higher dilution with an alcohol-treated suspension usually indicates that the patient has typhoid fever or an infection incited by a species allied to Bacillus typhosus, while a similar reaction with a formaldehyde-treated suspension suggests one of three alternatives, that the patient has typhoid fever, that he has had the disease in the past or that he has received typhoid vaccine. Both the granular and the floccular types of agglutination have seldom been observed in high dilutions of serum other than that from patients with typhoid fever.

FROM THE AUTHORS' SUMMARY AND CONCLUSIONS.

ATTEMPTS TO LOCATE THE SITE OF ANTIBODY PRODUCTION. G. A. H. BUTTLE, Brit. J. Exper. Path. **15**:64, 1934.

Some have thought that the reticulo-endothelial system is the site of the production of antibodies; others have been of the opinion that all tissues are involved. It has been reported that tissue cultures produce antibodies. The author injected into rabbits a 2 per cent alum-precipitated diphtheria toxoid and after a month

titrated the antitoxic power of the blood. Then an exsanguination and transfusion were done so that four fifths of the circulating blood was substituted by normal rabbit blood. It was found that the blood does not produce antitoxin, but acts merely as a vehicle in which it is carried around the body after it is formed by the tissues. Removal of the liver, spleen and skin did not affect the rate of antitoxin production in rabbits. Probably all the tissues take part in the production of antibodies.

JACOB KLEIN.

ABSORPTION OF ANTITOXIN THROUGH THE SKIN. R. RICHOU, Ann. Inst. Pasteur **51**:117, 1933.

Tetanus antitoxin in various forms was applied to the skin of guinea-pigs. Percutaneous absorption was manifested, but the skin appeared to receive antitoxin only passively, permitting some slight general absorption. Only about 1 part in 1,000 had any effect. "Between the effect of antitoxin introduced by one or the other of these ways there was no qualitative difference, but only quantitative differences, the disadvantage remaining, from many points of view, with the percutaneous method."

M. S. MARSHALL.

PERCUTANEOUS SERUM SENSITIZATION. R. RICHOU, Ann. Inst. Pasteur **51**:146, 1933.

Following percutaneous application of tetanus antitoxin guinea-pigs were found to be sensitized to serum to an extent permitting anaphylactic shock. Such sensitized animals were not desensitized by percutaneous application of serum a day before the test shock dose was given.

M. S. MARSHALL.

RED BLOOD CELLS AND IMMUNITY. R. DUJARRIC DE LA RIVIÈRE and N. KOSSOVITCH, Ann. Inst. Pasteur **51**:149, 1933.

Red blood cells adsorb diphtheria toxin; the adsorption capacity of the cells varies with the species of animal; cell stroma fails to fix toxin or fixes it in minimum quantity. Tetanus anatoxin fixed on red blood cells preserves them from the action of the corresponding toxin. The serum of animals immunized with a hemoglobin which has been submitted to several successive crystallizations possesses antibodies which corresponded to the antigen and which are rigorously specific.

AUTHORS' CONCLUSIONS.

ADENOCARCINOMA AND SQUAMOUS CELL CARCINOMA. J. NOCHIMOWSKI, Frankfurt. Ztschr. f. Path. **44**:547, 1933.

This is a review of the literature and a report on four additional cases. As to the pathogenesis of these tumors it appears that two possibilities must be considered. One is that both the adenocarcinoma and the squamous cell carcinoma, arising from different parts, are growing into each other and thus give rise to the formation of "collision tumor." It is also possible that such tumors may arise from cells of so little differentiation that they are capable of developing into both adenocarcinoma and squamous cell carcinoma. These rare tumors are classified by Nochimowski as "true" or "primary" adenocarcinoma and squamous cell carcinoma.

WILLIAM SAPHIR.

PECULIAR TUMOR ARISING IN THE GONADS OF AN INTERSEX. E. BRAUER, Frankfurt. Ztschr. f. Path. **45**:224, 1933.

An 18 year old person revealed a relatively small penis with hypospadias and absence of the prostate, seminal vesicles and testes. Because of a sudden attack of pain in the lower quadrant and accompanying fever, the patient was operated

on. A uterus and two tubes were found, and in place of the ovaries two large tumors, one of which appeared twisted and necrotic. The tumors and both tubes were removed. The patient died subsequently, and permission for a postmortem examination could not be obtained. Each tumor weighed over 3 pounds (1.4 Kg.). They were yellowish white and contained coarse nodules. The tubes histologically corresponded to those seen in females about 12 years old. The tumors consisted of connective tissue septums, between which heaps of cells were recognizable, with prominent nuclei. The cells were situated very close to one another and were surrounded by connective tissue which formed alveolus-like spaces. No blood vessels were seen within the tumors. The tumor cells were occasionally arranged in rows and islets, and sometimes presented a fanlike appearance. In some fields the connective tissue stroma and in others the epithelial elements predominated. This was so marked that Brauer concluded that the tumor was either an alveolar sarcoma or a carcinoma. Only a few lumens were seen, lined by large cuboidal epithelial cells.

O. SAPHIR.

GRANULOSA CELL TUMORS OF THE OVARIES. H. BETTINGER, Frankfurt. Ztschr. f. Path. **45**:238, 1933.

The first instance concerns a 56 year old woman who had suffered from irregular metrorrhagias. Examination of the specimen obtained by curettage led to a diagnosis of adenocarcinoma of the fundus. A panhysterectomy revealed a carcinoma in addition to two myofibromas; also that the left ovary was cystic and that the right showed many firm nodular tumors. The latter consisted of connective tissue fibers rich in nuclei with many intercalated fibers. Many large nests of cells were found within the stroma. The cells were polyhedral, containing small nuclei with much chromatin and a lightly stained cytoplasm. Only occasionally a slight tendency toward the formation of cysts was observed in the centers of the cellular foci. A second case was found in a 57 year old woman, who had a typical pseudomucinous cystoma in the left ovary. The right ovary contained a tumor similar to the one described in the ovary in the preceding case.

A second type of tumor was found in a 17 year old girl who had broad shoulders, small hips and absence of pubic hairs. The mammary glands were underdeveloped, and menstruation had not occurred. At operation, an infantile uterus was found, and a large solid tumor of the left ovary and a small tumor of the right were present. These tumors consisted of a connective tissue stroma with many lymphocytes; many foci of large cells with large round nuclei and much cytoplasm. No particular arrangement of the cells was noticeable. A sister of this patient, who had shown a similar tumor of the ovary, died two years after the operation as a result of generalized metastases.

O. SAPHIR.

RETICULAR LYMPHO-EPITHELIOMA OF THE THYMUS. E. LAAS, Frankfurt. Ztschr. f. Path. **45**:309, 1933.

An instance is described in a 62 year old man. The tumor was found beneath the sternum at the site of the thymus. It was well circumscribed within the fatty tissue and weighed 55 Gm. There were many spindle-shaped cells and lymphocytes situated within a network of reticulum fibers. Within the reticulum, mesenchymal cells but no tumor cells were present.

O. SAPHIR.

IMMATURE LOCALIZED, AND DIFFUSELY INFILTRATING RHABDOMYOBLASTOMA. M. GLASUNOW, Frankfurt. Ztschr. f. Path. **45**:328, 1933.

Six instances of rhabdomyoblastoma are described. Glasunow distinguishes between myoblastomyoma, which is a benign tumor, and myoblastosarcoma or diffusely infiltrating rhabdomyoblastoma, which are of the malignant variety. The so-called myoblastomyomas are well circumscribed, but not encapsulated, tumors.

They consist of round, oval or elongated cells measuring 15 to 30 microns in diameter. They reveal a slightly basophilic and finely granular cytoplasm. Also, syncytial masses are present, often arranged in the form of bundles. The stroma consists of thin collagen fibrils. Occasionally, the tumor elements may be very large. The malignant type is characterized by pleomorphism of cells. Giant cells are often encountered, and mitotic figures are present. The cytoplasm is distinct and finally granular. The finely granular cytoplasm is characteristic of the myoblastoma. Oxyphilic granules are occasionally found in the cytoplasm. A striation of the cytoplasm of the fibers may be encountered. The longitudinal striation is, as a rule, much more pronounced than the cross striation. There is no principal difference between the malignant myoblastomyoma and the rhabdomyoma.

O. SAPHIR.

MORPHOLOGY OF FAT IN CARCINOMA. B. KELLNER, Frankfurt. Ztschr. f. Path. **45**:383, 1933.

The fat content of fifty carcinomas was studied. Fat was found in the tumor cells and in the connective tissue. Squamous cell carcinoma, particularly the type which does not show keratinization, contained the least. Fat probably is carried to the tumor cells by the lymphatics or may be liberated by necrotic cells, but cannot be utilized by the neoplastic cells.

O. SAPHIR.

IMMUNE BIOLOGIC REACTION OF THE TRANSPLANTABLE MOUSE CARCINOMA. B. M. MÜHLENBEIN, Frankfurt. Ztschr. f. Path. **45**:514, 1933.

Mühlenbein succeeded in producing true anaphylactic reactions in white mice with hog serum. The animals were sensitized with hog serum (0.3 cc.) given at intervals of from three to seven days. Twenty-eight days after the last sensitizing injection, an intravenous administration of the serum was usually followed by death due to anaphylaxis. This method was utilized in an attempt to sensitize white mice with autogenous aqueous tumor extracts. While a true anaphylactic condition could not be brought about, it was found, nevertheless, that the sensitized animals showed a marked resistance against transplantation of the autogenous tumor. In cases of successful transplantation there was a definite retardation of the growth of the tumor.

W. SAPHIR.

IMMUNITY TO THE TRANSPLANTABLE MOUSE TUMOR. G. FISCHER, Frankfurt. Ztschr. f. Path. **45**:526, 1933.

Fischer attempted to produce immunity in white mice against transplantable tumors by preceding injections of human tumor material. Aqueous extracts of such material injected repeatedly into white mice did not produce an anaphylactic condition in the animals. The "taking" of transplantable mouse tumor in the treated did not differ from that in the control animals. Likewise, attempts to produce immunity by injections of the globulin fraction and the phosphatid fraction of human tumor extracts gave repeatedly negative results.

W. SAPHIR.

TUMOR GROWTH IN UNDESCENDED AND COMPENSATORY HYPERTROPHIC TESTICLES. E. DE BARY, Frankfurt. Ztschr. f. Path. **45**:556, 1933.

De Bary briefly mentions the theories of tumor origin and growth and includes that of Fischer-Wasels, who explains the origin and growth of tumors on the basis of a disturbance either of embryonal development or of regeneration and on Askanazy's "vegetative-activating" factor. According to Fischer-Wasels the anlage of a tumor arises only through a disturbance of embryonal or regenerative development (embryonal heteroplasia and regenerative metaplasia included). Askanazy maintains, however, that such an anlage might follow on a compensatory hyper-

trophic process. De Bary is evidently opposed to the latter view because, notwithstanding the frequency of compensatory hypertrophy of the heart, tumors of that organ are rather rare. Further, a kidney that has undergone compensatory hypertrophy shows a tendency toward inflammatory and degenerative changes rather than toward the formation of a tumor. In order to clarify the issue further he has investigated the incidence of neoplastic growth in undescended and compensatory hypertrophic testicles. A survey of the literature revealed only one questionable case of tumor in the normally placed partner of a cryptorchid testicle. On the other hand, he found, in accord with the literature, a far greater tendency toward the development of tumor in the undescended organ than in the scrotal hypertrophic organ. About 12 per cent of all undescended testicles reveal tumor formation. This tendency to the development of tumor is attributed to a disturbance in embryonal development.

H. HORN.

Medicolegal Pathology

DEATH FROM DINITROPHENOL. F. E. POOLE AND R. B. HAINING, J. A. M. A. **102**: 1141, 1934. FATAL DINITROPHENOL POISONING. M. L. TAINTER AND D. A. WOOD, J. A. M. A. **102**:1147, 1934.

Poole and Haining describe a case of death following the taking of 2,880 mg. of dinitrophenol in the course of five days by a woman 25 years old. A few hours before death the patient became restless, perspired profusely and complained of "burning up"; the temperature was at least 101.8 F.; the respirations were deep and rapid; death occurred in coma. No chemical tests were made of the urine. Examination of the body after death did not reveal any striking lesions of any kind; there were small submucous hemorrhages in the stomach and upper part of the small intestine. Microscopically the epithelium of the convoluted tubules showed various stages of degeneration, and in the liver the cells, especially at the periphery of the lobules, appeared to be separated by fluid. Dinitrophenol was demonstrated chemically in the kidney and liver.

The summary of the report of the second case, which occurred in a man, 37 years old, is as follows: Death occurred eleven hours following the oral administration of between eight and seven times the usual therapeutic dose of dinitrophenol. The dose taken was estimated by various methods to have been at least 2.4 but more probably 5 Gm. The man weighed approximately 80 Kg., giving an estimated dosage of 62.5 mg. of dinitrophenol per kilogram of body weight. The drug was self-administered in the apparent attempt to produce hyperpyrexia as a therapeutic measure for a supposed syphilis of the central nervous system. The rectal temperature shortly after death was so high that it could not be recorded by a clinical thermometer, probably being at least 115 F. The onset of rigor mortis was rapid, the body being rigid within ten minutes. A subicteric discoloration of the sclerae and conjunctivae was present and was due in all probability to the color of the drug itself. The anatomic changes consisted chiefly in a marked rigor mortis; an acute pulmonary congestion and edema; ecchymotic hemorrhages in the endocardium, pericardium and pia; mild nephrotic changes in the kidneys, and a slight detachment of the hepatic cells from one another. The clinical and anatomic changes bore a striking resemblance to those seen in cases of heat stroke.

CYTOLOGY OF BREAST SECRETIONS. M. KERUBACH AND C. COTUTZ, Deutsche Ztschr. f. d. ges. gerichtl. Med. **22**:235, 1933.

The cytology of the secretions of the breast after miscarriage or labor may be of aid in medicolegal diagnosis. The cell formula of these secretions renders possible the diagnosis of abortion or labor, regardless of retrogressive changes in the uterus. This diagnosis is possible up to three weeks after abortion and longer after labor, and is based on the finding of marked epithelial desquamation. Nonpregnant women show no epithelial elements in the secretions of the breast. There

is a preponderance of epithelial cells in the breast secretions of nonnursing women after pregnancy and after weaning. Tables and illustrations of the constituents of different types of mammary secretions are given.

JACOB KLEIN.

FAT EMBOLISM AFTER TRAUMA AND AFTER BURNING. GEORG STRASSMANN, Deutsche Ztschr. f. d. ges. gerichtl. Med. **22**:272, 1933.

Among 125 persons who succumbed to fatal trauma from blunt forces 8 died suddenly without evidence of fat embolism. However in 17 immediately fatal and 19 rapidly fatal cases fat embolism was demonstrable. When fat embolism was the cause of death there were associated fractures of the long bones, ribs or pelvis. Fat emboli were found in the lungs up to thirteen days after trauma. After burns fat embolism was rare and was mostly associated with bone trauma.

JACOB KLEIN.

MORPHOLOGIC CHANGES IN VISCERA DUE TO DIRECT CURRENT. JULIUS INCZE, Deutsche Ztschr. f. d. ges. gerichtl. Med. **22**:309, 1933.

The direct current causes specific morphologic changes in the parenchyma and stroma of the viscera. For example, the epithelial cells of the renal tubules may assume a characteristic elongated form.

JACOB KLEIN.

SPECTROSCOPIC DEMONSTRATION OF CARBON MONOXIDE IN BLOOD WITH THE AID OF SODIUM STANNITE. O. SCHMITT, Deutsche Ztschr. f. d. ges. gerichtl. Med. **22**:379, 1933.

To 5 cc. of blood 5 small drops of a freshly prepared sodium stannite solution were added, and the blood was examined under the spectroscope. By this means it was possible, as illustrated in the spectrographs, to demonstrate as little as 5 per cent carbon monoxide in the blood.

JACOB KLEIN.

SUCCINIC ACID IN CADAVERS. A. VERDINO, Deutsche Ztschr. f. d. ges. gerichtl. Med. **22**:384, 1933.

From two cadavers examined for organic poisons succinic acid was isolated in considerable amounts. From the one 60 mg. was isolated, from 807 Gm. of tissue; in the other 190 mg. was found in 1,886 Gm. of tissue. The presence of succinic acid is ascribed to bacterial putrefaction.

JACOB KLEIN.

ISO-AGGLUTININ IN OLD BLOOD STAINS. R. WITTE, Deutsche Ztschr. f. d. ges. gerichtl. Med. **22**:397, 1933.

Twenty-three blood stains ranging in age from five to fifty-five years were found to contain iso-agglutinins.

JACOB KLEIN.

SPECTROGRAPHIC EXAMINATION IN ELECTRICAL INJURIES AND GUNSHOT WOUNDS. W. GERLACH, Deutsche Ztschr. f. d. ges. gerichtl. Med. **22**:432 and 438, 1934.

This method of examination is of forensic significance because metallization of tissues and clothing in electrical accidents as well as the presence of various metallic substances in gunshot wounds can be demonstrated readily.

FORMALDEHYDE POISONING. K. BÖHNER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **23**:7, 1934.

A 40 per cent watery solution of formaldehyde causes severe poisoning even in small quantities. In the air passages inflammatory changes develop, with hemorrhage, cell infiltration and edema; in the digestive canal, fixation and hardening of the tissues, also necrotic gastritis and enteritis; in the kidneys, parenchymatous

degeneration. The chief symptoms are dyspnea and cyanosis, dysphagia and vomiting, dizziness and unconsciousness. Death appears to result from respiratory paralysis.

FROM THE AUTHOR'S SUMMARY.

EFFECT OF COLD AND LIGHT ON THE DETERMINABILITY OF THE BLOOD GROUP. B. MUELLER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **23**:40, 1934.

Experiments with blood of group A showed that freezing does not materially reduce the determinability of the group. This was the case whether the blood was exposed to cold in the fluid, partially dried or dried state. Sunlight or light from a quartz lamp may reduce the group characteristics materially.

Technical

COMPARISON OF ASCHHEIM-ZONDEK AND FRIEDMAN TESTS IN NORMAL AND ABNORMAL PREGNANCY. H. C. MACK AND G. H. AGNEW, Am. J. Obst. & Gynec. **27**:232, 1934.

Mack and Agnew compared the results of the Aschheim-Zondek test in 546 cases with those of the Friedman modification in 566 cases. The results were almost identical for practical purposes, but the simplicity of technic, the ease of interpretation and the factor of speed strongly favor the method of Friedman. Four cases of choriocarcinoma showed the well known abundance of eliminated hormone. The authors emphasize the need for correlation of the test with the clinical picture, as under circumstances, a negative test may be compatible with an interrupted pregnancy with the products of conception still present in the uterus or tube, and on the other hand, a positive test may be present soon after a termination of pregnancy or in certain abnormal conditions (choriocarcinoma, for example).

I. DAVIDSOHN.

A NEW METHOD OF READING THE FRIEDMAN MODIFICATION OF THE ASCHHEIM-ZONDEK TEST. M. DAVIS, W. KONIKOV AND ELISABETH M. WALKER, Am. J. Obst. & Gynec. **27**:274, 1934.

The method is based on the observation of Bercovitz that following the instillation of blood serum of pregnant women into their own conjunctival sacs there follows a pupillary reaction which is absent in nonpregnant women. Davis and his associates observed contraction or dilatation of the pupils in rabbits immediately following the intravenous injection of the urine of pregnancy. The results were compared with the usual changes in the ovaries. There was agreement in 90.6 per cent of the cases of pregnancy, and in 81.8 per cent of the cases of nonpregnancy.

I. DAVIDSOHN.

CONGENITAL SYPHILIS FROM A TRANSFUSION OF BLOOD TO MOTHER DURING PREGNANCY. G. R. WILLIAMSON AND R. A. STRONG, Am. J. Syph. & Neurol. **7**: 484, 1933.

A married nurse, four months pregnant, presented cellulitis of her right arm and septicemia, both of which developed in the course of her work, and which necessitated a transfusion of blood. Two transfusions were given, though no Wassermann test was made of the donor. Six weeks later secondary syphilis developed in the patient. There was no reason to suspect syphilitic infection prior to the transfusion. The husband and first child were perfectly normal. This pregnancy resulted in the somewhat premature birth of a 5 pound (2,268 Gm.) congenitally syphilitic infant. The literature is reviewed. The apparent inadequacy of precautions taken in many such cases indicates the need for greater vigilance. At present there is added risk because a less desirable class of professional donors are offering themselves, owing to unemployment in other fields.

JACOB KLEIN.

A Simple Instrument for Use on the Vertebral Column. F. Henschen, Centralbl. f. allg. Path. u. path. Anat. **60**:55, 1934.

Henschen describes a powerful, slightly curved chisel, 25 cm. long, with a cutting edge of 8 cm., an eight-cornered handle and a large flat head. The instrument is made of one piece of steel and is driven into the vertebrae with a wooden or hard rubber mallet. The advantages claimed for this device are: its simplicity and ease of manipulation; the clean cuts free from sawdust, and the possibility of removing the spinal cord from the front.

George Rukstinat.

A Simple and Rapid Chemical Hormonal Pregnancy Reaction. E. Cuboni, Klin. Wchnschr. **13**:302, 1934.

The urine of the pregnant mare contains so little of the hormone of the anterior lobe of the pituitary gland that it cannot be used for the diagnosis of pregnancy by means of the well known reaction in infantile female mice. On the other hand, the urine of the mare contains abundant quantities of follicular hormone. The diagnostic test for pregnancy is based on the injection of the urine into infantile rats or mice, or into castrated mice, and on the observation of a characteristic desquamation of vaginal epithelium. Kober reported (*Biochem. Ztschr.* **239**:209, 1931) the appearance of fluorescence when heated follicular hormone was treated with concentrated sulphuric acid. Cuboni applied that to the diagnosis of pregnancy in the mare. The urine is filtered through filter paper; to 5 cc. of it, 1 cc. of concentrated hydrochloric acid is added and the mixture is heated for ten minutes on a boiling water bath, then it is cooled under running water and 6 cc. of benzene is added. After vigorous shaking (fifty times), the urine is discarded and the supernatant benzene is permitted to settle. Then 3 cc. of the benzene extract is dried by heating at 60-80 C. The sediment is dissolved completely in 0.8 cc. of concentrated sulphuric acid. The solution is heated for a few minutes at 70-80 C. If the test is positive a green fluorescence is shown when the solution is viewed in reflected light. In transmitted light the appearance is (dark red, brown-red or brown) in pregnancy and in nonpregnancy. The performance of the test takes from fifteen to twenty minutes. Another procedure consists in adding 1 cc. of concentrated sulphuric acid to the benzene extract. The mixture is heated for a few minutes on the water bath at 70-90 C., shaken and left for five minutes. Under the colorless layer of benzene there is a layer of sulphuric acid which shows fluorescence in cases of pregnancy. The reaction was correct in all thirty-five cases of pregnancy of mares and in numerous negative controls. The addition of preservative (phenol) did not interfere with the test. Non-preserved urine was satisfactory for the test for more than a month when kept in the icebox, and for fourteen days at 37 C. Specimens which were inoculated with Bacillus proteus could also be used. A similar technic permits the carrying out of the test with blood serum.

I. Davidsohn.

The Reliability of the Friedman Rapid Test for Pregnancy. F. Hein, München. med. Wchnschr. **80**:1687, 1933.

Hein found the Friedman test quite as reliable as the original Aschheim-Zondek test with the additional advantages of greater speed and easy interpretation. He found, however, that in seven of sixty-five cases, or in 11 per cent, the result was still negative after twenty-four hours, but was strongly positive when read after forty-eight hours. He advises the use for each test of two animals weighing about 2,000 Gm. One of them can be examined after twenty-four hours and if the result is positive, no further observation is necessary, but if the result is negative, the second animal is to be examined twenty-four hours later, at which time the result is final, whether positive or negative.

I. Davidsohn.

Society Transactions

AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

C. PHILLIP MILLER, *Secretary*

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THE METEOROLOGICALLY CONDITIONED BIOLOGIC RHYTHM AND EXPERIMENTAL PRODUCTION OF ENDOCARDITIS. WILLIAM F. PETERSEN and A. J. NEDZEL (by invitation), University of Illinois.

When patients are followed day by day clinically or chemically, a definite phase difference in every process can be observed. A phase of decreased activity (A R S) is followed by one of increased activity (C O D). During the former the organism is more alkaline, anabolism preponderates, and with this, reduction is enhanced and spasm is more pronounced in the smooth musculature (high blood pressure, etc.). This phase leads to a definite anoxemia, which in turn initiates the C O D phase, when catabolism, oxidation, increased metabolic rate, relative acidity and dilatation of the blood vessels are all more pronounced. The normal organism pendulates between two such poles. The unstable person reveals evidence of greater amplitude in these rhythmic waves.

The blood pressure curve illustrates the rhythm very well and affords a simple method of observation; periods of pressor increase are followed by distinct lowering of the diastolic blood pressure, not infrequently cumulative and sustained (C O D phase).

During the pressor phase certain tissues of the body become relatively anoxemic, and this in turn causes stimulation, with a distinct change in the functional status. Regions of potential vascular insufficiency (terminal vessels, epithelial tissues, tissues with sclerotic impairment) are of necessity more often affected than others.

The rhythm is conditioned by many factors—endocrine, emotional, infectious—but perhaps the most important environmental alteration in this region of the world is the meteorological. As a result the changing tide of the A R S and C O D phases is induced largely by meteorological alterations (that is, the cyclonic circulation of the atmosphere). Pressor events are usually associated with a cold or polar front (they may occur, however, with high humidity and heat), and as a result with clinical episodes in the patient—that is, the precipitation of disease is often intimately related to the pressor episodes and the passage of polar fronts.

Inasmuch as bacterial endocarditis is so distinctly seasonal in its onset we have sought to determine whether bacterial localization on the heart valves might follow artificially induced pressor episodes (pitressin). In a series of dogs, streptococci and staphylococci intravenously injected were found to localize on the valves when the animals had been previously given injections of pitressin. Lesions so induced were typical of the ulcerative endocarditis and, in some instances, of the vegetative type of endocarditis that are seen in man (demonstration). The increased demand caused by increased pressure is apparently associated with increased permeability and stickiness of those portions of the valve that come into contact more forcibly, and in such regions bacteria are more liable to localize.

Not only will pressor events, therefore, have the tendency to permit the passage of the bacteria present in the mucous membranes through these mem-

branes, but the same meteorological event or a succeeding one will enhance the possibility of localization of such bacteria on a valve—or in other regions of the body that have been subjected to anoxemic stimulation (spinal cord, giving rise to poliomyelitis; joints, inducing arthritis; dental roots, gallbladder, etc.).

HEMORRHAGIC REACTIONS IN NORMAL AND TUBERCULOUS ANIMALS. JULES FREUND, Cornell University.

When certain toxic bacterial products are injected into the blood stream of tuberculous guinea-pigs, the skin at the site of a positive tuberculin reaction becomes hemorrhagic.

In normal guinea-pigs, tuberculin does not prepare the skin to a hemorrhagic reaction.

Tuberculin does not produce an intense or necrotic inflammation in the skin of tuberculous rabbits and fails to prepare the skin for the hemorrhagic reaction.

Nontuberculous guinea-pigs sensitized to horse serum react to horse serum injected into the skin, with redness and edema. Such a reaction is not influenced by a subsequent injection of a potent bacterial filtrate. Tuberculous guinea-pigs sensitized to horse serum react to undiluted horse serum with redness and edema followed by necrosis; the necrosis is sometimes preceded by purple discoloration, an observation described by Dienes. When tuberculous guinea-pigs sensitized to horse serum are given an intradermal injection of diluted horse serum, their reaction becomes hemorrhagic after subsequent injections of bacterial filtrates into the circulation.

As a preparatory factor, diphtheria toxin acts regularly in the normal guinea-pig; silver nitrate, in some of the nontuberculous and in a large proportion of the tuberculous guinea-pigs. Turpentine, broth and aleuronat are inactive in both tuberculous and nontuberculous guinea-pigs.

As factors producing injury, filtrates from meningococci, typhoid bacilli and colon bacilli are active in sublethal doses. Tuberculin is active in tuberculous guinea-pigs when it is used in lethal doses. Witte's peptone, agar and starch produce anaphylactoid symptoms but are as a rule inactive as injury-producing agents.

In the guinea-pig, the skin-preparatory agents have a tendency to produce hemorrhages even without the subsequent injection of the injury-producing agent, and this property may be essential in their action. The production of symptoms of anaphylactoid shocks is not always associated with the production of hemorrhage, as shown by experiments with Witte's peptone, agar and starch.

TUBERCULOSIS IN SYPHILITIC AND NONSYPHILITIC RABBITS. JOSEPH D. ARONSON and DAVID R. MERANZE (by invitation), The Henry Phipps Institute, and Mount Sinai Hospital, Philadelphia.

Tuberculous lesions induced by the injection of 0.1 mg. of a culture of the bovine type of tubercle bacillus into the skin of syphilitic and nonsyphilitic rabbits have been studied. Tubercle formation was accelerated and progressed more rapidly in the syphilitic rabbit. Twelve hours after inoculation polymorphonuclears occurred in the tissue about the site of injection as definite discrete aggregations whereas in the nonsyphilitic rabbit polymorphonuclear infiltration was diffuse throughout the subcutaneous tissue. From twenty-four to forty-eight hours after inoculation discrete aggregations of large mononuclear cells with pale-staining nuclei were noted about the site of injection in the syphilitic rabbit. These cells tended to increase, as did the fibroblasts. In the nonsyphilitic rabbit a large, single, circumscribed lesion consisting of polymorphonuclear cells was noted within the first week following infection. In both groups of animals surface ulceration occurred about the second or third week. In the syphilitic rabbit granulation tissue was noted beneath the ulceration, and epithelioid cells were aggregated in groups surrounded by connective tissue. In the nonsyphilitic rabbit the granulation tissue was minimal, caseation was marked, and extensive epithelioid prolifera-

tion occurred beneath the ulcer. Caseation was less marked in the syphilitic rabbit, granulation tissue was more marked, and epithelioid cells occurred as discrete aggregations, while in the nonsyphilitic rabbit the epithelioid cells occurred in extensive diffuse areas.

OCCURRENCE OF LESIONS IN RABBITS FOLLOWING INJECTION WITH BCG. WILLIAM H. FELDMAN (introduced by F. C. MANN), The Mayo Clinic.

A strain of BCG obtained from Calmette in 1930 and subsequently grown for twenty generations on an egg-glycerin medium was transferred to glycerin-peptone-broth medium and the cultures used to inoculate a series of six rabbits intravenously and four guinea-pigs subcutaneously. One of the rabbits died ten days after inoculation and the other five were killed after one hundred and seventy-four days. Numerous and striking tubercle-like lesions occurred in the lungs of each of the five rabbits. Attempts to cultivate acid-fast bacteria from the lesions were futile, although bacteria of this character were readily demonstrable in appropriately stained sections of the lesions. Emulsions of tissue showing the lesions taken from each of the five rabbits failed to produce demonstrable lesions in other rabbits or guinea-pigs, and attempts to repeat the results in additional experiments have failed.

THE FATE OF BCG AND THE ASSOCIATED HISTOLOGIC CHANGES IN THE ORGANS OF RABBITS. MAX B. LURIE (introduced by ESMOND R. LONG), The Henry Phipps Institute, Philadelphia.

Three series of rabbits were inoculated intravenously with 1 mg. of a culture of a strain of BCG obtained from the Pasteur Institute. At various intervals organs, blood and bile were cultured on Löwenstein's egg medium modified by the addition of bone marrow extract. Tissues adjoining those cultured were studied histologically.

The greater the primary deposition of bacilli the more rapid was the initial growth of the bacilli and the earlier the beginning of their destruction. With the smallest original deposition, however, destruction began immediately in the lung and the liver, but at the same time multiplication occurred in the spleen and the lymph nodes, indicating local immunity of the lung and the liver.

The growth of the bacilli is associated with a local multiplication of mononuclears by mitosis, their accumulation into nodules, the destruction of the bacilli and a formation of epithelioid and giant cell tubercles. Necrosis of invading polymorphonuclears, exudation of fluid and cells into the alveoli and caseation occur with the development of hypersensitiveness to tuberculin. By the fourth week, when this was marked, bacilli had been almost completely destroyed in all organs and lymph nodes.

All the tuberculous changes were usually resolved completely by the second month; yet isolated bacilli persisted in lymph nodes even fourteen months after inoculation.

EFFECT OF FERRIC CHLORIDE IN EXPERIMENTAL TUBERCULOSIS. VALY MENKIN, Harvard Medical School.

Previous studies had shown that repeated intravenous injections of 0.25 per cent ferric chloride in rabbits infected with bovine tubercle bacilli were followed by an accumulation of iron in the tubercles (chiefly within the caseous areas). Concomitantly there was a retardation in the development of the disease, as evidenced both by an increase in the survival time and by a less extensive tuberculous involvement in the treated as compared with the nontreated animals. These results were obtained in two independent series of experiments.

These studies have been continued. In the present series virulent bovine tubercle bacilli (Ravenel) were inoculated subcutaneously instead of intravenously as in the previous experiments. Treatment with ferric chloride was started *promptly* after

inoculation of the bacilli and was continued for about four months. Five controls lived from 91 to 170 days, with an average survival time of 130 days. Four experimental rabbits survived from 112 to 326 days. One experimental rabbit was killed 344 days after inoculation. The average longevity of the experimental rabbits was 246 days, an increase of 89 per cent over the survival time of the controls.

Exactly the same type of experiment was repeated in ten rabbits which, however, had been previously inoculated by intravenous injection of a relatively avirulent strain of bovine tubercle bacilli (Cernay), in order to see whether partial immunity induced by the previous infection would enhance the retarding effect of ferric chloride. Such data would be of importance in reference to adult human tuberculosis, which is doubtless the tuberculosis of reinfection. The five controls lived between 47 and 130 days, with an average survival time of 81 days. Three of the experimental rabbits died 81, 110 and 131 days after subcutaneous reinoculation with virulent tubercle bacilli. Two of these animals succumbed to infections of the upper respiratory tract. The two remaining experimental rabbits progressively increased in weight and lived for over six months after the death of the last control animal. On the three hundred and thirty-fourth day after reinoculation they were killed. The extent of the tuberculous involvement in these two animals was far below that found in the last survivors of the control group. The average longevity of the experimental rabbits was 198 days, an increase of 144 per cent over the average survival time of the controls. By employing the subcutaneous route of infection and instituting intravenous ferric chloride treatment *immediately,* more pronounced effects were obtained than in the previous series. In vaccinated animals the results seemed to be even more striking than in the nonvaccinated group.

The mechanism of the favorable effect of ferric chloride is now under investigation. The frequent finding in the lungs of treated animals of zones of engorged capillaries about tubercles, at times associated with some fibroblastic proliferation, suggests the end-stage of a superimposed inflammatory reaction induced by the accumulation of iron in the tubercles. Ferric chloride is in itself an intense inflammatory irritant. The lungs of animals surviving long after the cessation of treatment were often characterized by moderate connective tissue proliferation at the peripheries of tubercles. These fibrous zones, in their location with respect to the tubercles, corresponded in a general way to the areas of engorgement found at an earlier stage. Similar areas of congestion were observed in a comparatively small percentage of the control tuberculous rabbits. The hypothesis is being subjected to further study.

It is noteworthy that in experimental rabbits, hemosiderin, or at any rate a substance indistinguishable from it by the usual tests, is found in great abundance in granular form within the mononuclear phagocytes of the spleen, in the Kupffer cells of the liver, in the reticular cells of the bone marrow, within the tubules of the kidney and sometimes in the cells of the alveolar walls in the lungs. These animals had no anemia. This raises the question whether hemosiderin is necessarily a product of the partial degradation of hemoglobin, or whether the mere cellular release of iron, which in turn may combine with some of the plasma proteins, is not sufficient to account for the formation of hemosiderin. Furthermore, it is conceivable that the hemosiderosis observed in experimental animals may also play a part in the favorable effects of ferric chloride on the course of tuberculosis in rabbits by activating and causing a new formation of cells of the reticuloendothelial system. That this may be is suggested by the following observations: Variable degrees of fibrosis at the peripheries of malpighian follicles were found associated with extensive hemosiderosis in the spleen. In the pulmonary lesions of the experimental rabbits many mononuclear cells at the peripheries of caseated centers were occasionally seen to be loaded with iron-staining material. Two normal rabbits that had received repeated intravenous injections of 0.25 per cent ferric chloride for one month were subsequently inoculated intravenously with

virulent bovine tubercle bacilli. They survived 115 and 124 days respectively, while seven tuberculous rabbits that had received no such preliminary treatment survived on the average 54 days, the longest survivor dying on the sixty-seventh day of its disease. This subject is now being investigated further.

EXPERIMENTAL PULMONARY EMBOLISM AND INFARCTION. BERNHARD STEINBERG and CARLL S. MUNDY (by invitation), Toledo Hospital, Toledo, Ohio.

Pulmonary emboli were produced in dogs by introduction of lead shot into their jugular veins. At varying intervals the emboli were followed by injections of iodized poppy-seed oil 40 per cent. Complete closure of the lumen of the vessel was produced. Hemorrhagic infarcts appeared involving parts or the whole of a lobe. These infarcts at the sixth or seventh day began gradually to disappear with a return of the tissue of the lung to an approximately normal state except for moderate interstitial fibrosis. At varying intervals following the establishment of pulmonary embolism, iodized oil was injected into the bronchial circulation. During the period in which the infarcts were most prominent, the bronchial circulation in the infarcted areas was not very apparent. On the fifth day, the bronchial circulation became significant, and coincidentally with the return of the infarcted lung to a comparatively normal state the bronchial circulation was very prominent, resembling in extent the pulmonary circulation. Although the present experiments do not establish either the independent blood supply or the free anastomosis between the two circulations, indirect evidence points to the bronchial arteries as independent in the assumption of the circulation in the infarcted area.

Although as many as three hundred and fifty shot were introduced into the pulmonary circulation and the dogs were kept for a year and a half, at no time was there any untoward symptom or death due to embolism.

THE MOVEMENT OF PARTICULATE MATTER ON THE MUCOUS SURFACES OF THE TRACHEA AND BRONCHI. R. Z. SCHULZ (introduced by S. B. Wolbach), Harvard Medical School.

The transportation of minute particles of carbon and garnierite in the trachea and bronchi has been observed in guinea-pigs, rabbits, cats, dogs and chickens. The general movement is from the bronchi toward the larynx with a slow and, at times, imperceptible movement in the bronchi and a more rapid propulsion in the trachea. The rate at which particulate matter is carried along the mucous surfaces varies with the different species tested, being slowest in guinea-pigs and chickens and increasingly more rapid in rabbits, cats and dogs. The factors which appear to influence the rate of movement are the amount and the tenacity of the film of mucus or fluid and the activity of the cilia. In animals infected with Streptococcus haemolyticus or Bacillus bronchisepticus, in which the exudate is of a seropurulent type, the rate of movement is greatly increased. Alcohol, ether and phenobarbital sodium, in narcotizing doses, do not appear to alter the activity of the cilia. Chloroform causes a slowing and early cessation of activity.

These studies indicate that one of the important functions of the ciliated cells of the trachea and bronchi of the mammal is to facilitate the mucous glands in maintaining an even film of mucus on the lining epithelium. A second function is to aid in the elimination of particulate material from the tracheobronchial tree.

AN ATYPICAL REACTION TO VACCINE VIRUS IN THE RABBIT. LOUISE PEARCE, C. K. HU (by invitation) and PAUL D. ROSAHN (by invitation), The Rockefeller Institute for Medical Research, New York.

The reappearance of rabbit pox last December approximately ten months after the subsidence of a severe epidemic of the disease necessitated the immediate application of methods designed to protect a rabbit-breeding colony of some one thousand and eight hundred animals. A considerable number of the older rabbits had recovered from the disease and might still be immune, but there were many

young adults and a large number of young born after the epidemic which presumptively were susceptible. On the basis of the results of experiments in which it was found that immunity to vaccine virus afforded some protection against rabbit pox, the colony was vaccinated with New York City Board of Health virus which had been propagated in tissue culture by Dr. T. M. Rivers. The method of vaccination employed was the intradermal injection of virus at a single site.

This mass vaccination resulted in several types of reactions which, according to generally prevalent opinion, are atypical. They occurred so frequently, however, as to raise the question whether current conceptions of vaccinial reactions are sufficiently inclusive.

In the susceptible adults, the expected local reaction developed, and in a number of cases a few scattered papules in the shaved skin surrounding the site of vaccination were observed. But, in addition, a generalized papular eruption occurred not infrequently, the lesions being distributed in portions of the body remote from the local reaction, for example, the ears. Other symptoms, such as generalized adenitis and orchitis in male animals, were common. Many of these rabbits were obviously ill, and some of them critically so, but there were only three fatalities.

In the young stock, all presumably susceptible, the results were sharply divided into two classes, one comprising typical local reactions to vaccination, and the other, results in which no local reaction was seen. In the first group there were a number of reactions associated with generalized eruptions corresponding to that which obtained among the adults, but the condition was in general more severe and the mortality considerably higher. In the rabbits in which no local vaccinial reaction was observed generalized lesions were comparatively frequent and severe and the death rate was high. Furthermore, there were several fatal cases in which no clinical evidence of vaccinial infection had been observed, either local or general, but in which postmortem examination revealed a more or less widespread distribution of vaccinial lesions in the viscera. Finally, there remained a group of young, presumably susceptible rabbits which gave no clinical indication, either local or general, of any reaction to the vaccination.

There are many aspects of these results which should be briefly referred to since the situation is by no means a simple one. The character of the reaction in nursing young was apparently influenced by the immune or nonimmune state of the doe, and the reaction of different breeds was not identical. There was likewise a sex difference, the reaction being generally milder in females than in males and especially so in pregnant and nursing does. The relation of the type of reaction to the size of the dose of vaccine virus administered must also be considered. These and other points as well must be taken into account before a final appraisal of the results can be made.

It is evident, however, that the results of our experience do not support the opinion that in the rabbit a wide distribution of lesions after local vaccination is unusual. Generalized vaccinia may be frequent, at least in certain circumstances, and, furthermore, the clinical manifestations of the condition are sufficiently distinctive to differentiate it as a disease entity.

SPORADIC ENCEPHALITIS IN COWS. F. S. JONES and RALPH B. LITTLE (by invitation), The Rockefeller Institute for Medical Research, Princeton, N. J.

Since 1930 a disease clinically characterized by well defined nervous symptoms has been studied. The brains of thirteen affected cows have been obtained. In all instances except one the animals were slaughtered between the second and sixth days of the attack. The incidence in the herd has been low, but a single case is apt to occur in a given group over a period of years. Adults of both sexes are affected.

Gross lesions either in the viscera or in the central nervous system have not been observed. Examination of fixed and stained material reveals well defined changes localized principally in the midbrain, stem and anterior portion of the cord. These consist in tiny areas of softening. Early in the disease the lesions consist of a

loosely arranged accumulation of polymorphonuclear leukocytes; later the polymorphonuclears become more numerous, and round cells appear. When the disease is well advanced the lesions are principally composed of round cells. Well defined perivascular infiltrations are encountered in the vicinity. These infiltrations, like the areas of softening, change in character and finally become almost purely round-celled. Cortical and meningeal lesions are infrequent.

Although the disease resembles others caused by filtrable agents, thus far it has not been possible to demonstrate such an agent. The inoculation of suspensions of affected midbrain and stem intracerebrally into calves, rabbits or guinea-pigs results in an acutely fatal meningitis. From both the original material and the brains of the experimental animals a gram-positive rod is readily cultivated. A similar organism is readily identified in the earliest lesions of the disease (microscopic areas of softening) in the fixed and stained preparations.

Intracerebral inoculation of calves, guinea-pigs and rabbits with a pure culture of the organism as a rule results in fatal meningitis. When extremely small doses are given, or the animal's resistance is stimulated by a previous intranasal inoculation, encephalitis may result.

THE PATHOGENICITY OF SWINE INFLUENZA VIRUS FOR FERRETS. RICHARD E. SHOPE (introduced by Carl Ten Broeck), The Rockefeller Institute for Medical Research, Princeton, N. J.

The observation of Smith, Andrewes and Laidlaw that the virus of swine influenza is pathogenic for ferrets when administered intranasally has been confirmed. A disease that is clinically more severe and pathologically more extensive than that described by the aforementioned workers is obtained if the ferrets are anesthetized with ether prior to infection. Animals infected in this way show at autopsy an edematous type of pneumonia of lobar distribution which may sometimes terminate fatally. The virus is easily transmitted serially through ferrets, and it maintains its pathogenicity for this species when stored in 50 per cent glycerol at refrigerator temperature for as long as seventy-five days. After serial passage through twelve ferrets, the virus is still capable of inducing swine influenza when mixed with Haemophilus influenzae-suis and administered intranasally to swine. Passage through ferrets causes no apparent attenuation of the virus for swine. Serum from pigs recovered from swine influenza is capable of neutralizing the ferret-passed virus for either swine or ferrets. Likewise, serum from recovered ferrets neutralizes the swine influenza virus for either ferrets or swine.

MATERNAL TRANSMISSION OF VACCINIAL IMMUNITY IN SWINE: II. THE DURATION OF ACTIVE IMMUNITY IN THE SOW AND OF PASSIVE IMMUNITY IN THE YOUNG. JOHN. B. NELSON (introduced by Carl Ten Broeck), The Rockefeller Institute for Medical Research, Princeton, N. J.

Two sows which had previously been vaccinated with vaccinia virus have continued to transmit immunity to the suckling young of six successive pregnancies over a period of three years. Pigs from the sixth litter were as well protected as those farrowed at the end of the first pregnancy after vaccination.

Vaccination of the suckling young at successive age intervals showed that the maternally acquired immunity began to decline during the second month of life and in most cases had practically disappeared by the end of the third month. The virus used in testing the suckling pigs, during the first week after birth, exerted little or no immunizing effect.

THE KUPFFER CELL IN RELATION TO IMMUNITY TO THE VIRUSES. J. W. BEARD (by invitation) and PEYTON ROUS, the Rockefeller Institute for Medical Research, New York.

Kupffer cells containing particles of iron have been collected with the magnet and tested for their influence on the virus of vaccinia. The living cells neutralized

the virus, whereas killed ones were without effect. Similar findings were obtained with the clasmatocytes of aleuronat exudates, not only with the virus of vaccinia but with that causing the Shope fibroma. Polymorphonuclear leukocytes, on the other hand, whether alive or dead, were without any neutralizing effect on vaccinia, and living cells of this sort seemed to enhance the activity of the Shope virus.

THE HIGH PATHOGENICITY OF A RECENTLY ISOLATED STRAIN OF SPIROCHAETA PALLIDA. C. K. HU (introduced by L. Pearce), The Rockefeller Institute for Medical Research, New York.

A strain of S. pallida isolated in 1931 from an inguinal lymph node of a Chinese patient suffering from a profuse maculopapular syphilitic eruption was found to be highly pathogenic in its early passages in the rabbit. The clinical manifestations of disease induced by this strain in the first three passages were found to be of the same order as those of older strains which had been propagated in the rabbit for hundreds of generations.

Two experiments were carried out in March and October 1932, at which time the strain was in the fourth and sixth generations following passage through animals. In each experiment ten male rabbits were inoculated intratesticularly and ten in the skin of the prepuce. The results of the two experiments were practically identical. Furthermore, comparable results were obtained by the two different routes of inoculation.

The pathogenicity of the new strain was measured by the incidence, the time of occurrence and the character of the primary lesions, together with the incidence, the time of occurrence, the distribution and the character of generalized lesions including metastatic orchitis and lesions of the skin, periosteum and bones, and eyes. The results obtained in thirty-six of the forty animals that survived the observation period of three and a half months may be summarized as follows: The incidence of primary lesions was 100 per cent, with an average incubation period of 18.3 days; metastatic orchitis in the uninoculated testicle occurred in 88.9 per cent of the animals in an average period of 52.2 days after inoculation; the average relative incidence of generalized lesions was 7.4 lesions per animal; the average period of activity of the lesions, as measured by the difference between the mean time of the first and that of the last lesion, was 23.5 days.

These results were entirely comparable with those obtained in similar experiments carried out at the same time and under the same experimental conditions. In these experiments, four strains of S. pallida were used, namely, the Nichols, the Zinsser-Hopkins, and two older Chinese strains, all of which have been carried in rabbits for many years.

It would appear, therefore, that the capacity of a strain of S. pallida to induce in the rabbit the characteristic disease picture of experimental syphilis including a diversity of generalized manifestations is not necessarily dependent on a long sojourn of the strain in the new host, in which circumstances it has been presumed to have become "adapted" and thereby to be capable of the production of disease.

ARSENOXIDE (META-AMINO-PARA-HYDROXYPHENYL ARSENOXIDE) IN EXPERIMENTAL ANIMALS. O. M. GRUHZIT, Parke, Davis and Company, Detroit.

The therapeutic and the toxic effects of the arsphenamines are thought to be due to the action of their "breakdown" product, the arsenoxide (meta-amino-para-hydroxyphenyl arsenoxide), known under the specific name of mapharsen. The compound has been recently introduced in the treatment of spirochetosis. Its toxicologic and pathologic effects are presented here in brief.

The tolerated dose of mapharsen is from 10 to 22 mg. per kilogram according to the animal used. In the white rat the intravenous tolerated dose is from 18 to 20 mg. per kilogram. Rabbits tolerate from 10 to 14 mg. per kilogram. In rats, rabbits and dogs, an intravenous dose of from 1 to 2.5 mg. per kilogram causes no gross or microscopically demonstrable lesions in organs. A dosage of 3, 4 and 5 mg. per kilogram a week, or a total of 76 mg. per kilogram in a period of

fifteen weeks, in dogs causes occasional temporary albuminuria, but no organic changes such as result in abnormalities of the total nonprotein nitrogen or sugar content of the blood.

A dosage of from 6 to 8 mg. per kilogram causes albuminuria, mild congestion of the liver and cloudy swelling of the cortical tubular epithelium of the kidneys. The maximal tolerated and the minimal lethal dose (60 per cent respectively of the animals live or die) may induce from mild to severe changes in the kidneys and mild injury in the liver, spleen and other organs. The predominating changes in the organs of rabbits from the maximal tolerated and minimal lethal doses appear in general as follows:

At the end of twenty-four hours following a treatment all organs show moderate degrees of congestion. No focal hemorrhages or necrosis are present in the liver. The liver appears edematous; some of the hepatic cells are granular, and some appear partly lysed. The kidneys are congested and edematous and some tubules of the cortical zone show coagulation necrosis. The glomerulae appear congested.

At the end of seventy-two hours the liver is somewhat congested and edematous, but no focal necrosis or hemorrhage is noted. In the kidneys the coagulation necrosis is distinct and is limited to the cortical zone.

At the end of from five to seven days the pathologic changes have reached their maximal development. The liver appears almost normal. The kidneys show in place of the coagulation necrosis an extensive deposit of calcium in the tubules of the cortical zone. The glomerulae appear congested; the distal collecting tubules may contain casts, but otherwise the collecting tubules appear well preserved. The lungs of animals dying at this stage are congested, and the alveoli are filled with serous exudate. No coagulation thrombi are noted.

As the duration of life of the animal extends beyond the seven day period, the reparation of the tissue begins to take place, namely, decalcification in the kidneys. On the twelfth day the deposits of calcium have receded to include only the intermediate zone of the cortex. At the end of twenty-four days almost all the calcium has disappeared from the tubules, and fibrosis has taken the place of the necrotic tubules. At the end of thirty-six days the kidneys in a number of animals appear almost normal, except for increased fibrosis and a reduced number of tubules. In other animals the necrotic tubules have been replaced by deposits of fat.

In summary, mapharsen causes only slight injury to the liver even with toxic doses. It is strongly nephrotoxic in minimal lethal doses and in general causes coagulation necrosis of the cells of the cortical tubules. The necrosis is followed by deposition of calcium which in turn, on healing, is replaced by fibrosis or fat. The clinical dosage of from 1 to 1.5 mg. per kilogram causes neither histologic changes in the organs nor disturbance in the constituents of the blood.

The histologic changes, in general, resemble those produced by the arsphenamines, except that the liver at no time appears so severely injured as in the case of the latter.

FURTHER STUDIES ON PREVENTION OF SERUM SICKNESS. MOYER S. FLEISHER and LLOYD R. JONES (by invitation), St. Louis University.

Treatment of whole serum or of the pseudoglobulin fraction of serum by lipid solvents may markedly diminish its activity in causing serum sickness in rabbits. This treatment does not alter the solubility of the dried protein in physiologic solution of sodium chloride when special consideration is given to the choice of solvents and to the factors of time and temperature.

Studies have been made of the effect of this treatment of serum or serum fraction on its antibody content.

The factor or factors extracted by the lipid solvents have been investigated.

TREATMENT OF TYPHOID FEVER WITH ANTITYPHOID SERUM. GREGORY SHWARTZMAN, GEORGE BAEHR (by invitation) and WILLIAM Y. HOLLINGSWORTH (by invitation), Mount Sinai Hospital, New York, United States Marine Hospital, New Orleans, and Charity Hospital, New Orleans.

The phenomenon of local skin reactivity to bacterial filtrates made possible a demonstration of Bacillus typhosus toxic substances which are identical or closely related to true exotoxins and also made it possible to develop antitoxic serum which neutralized these toxic substances specifically and in multiple proportions. Serums of high neutralizing and agglutinating titers prepared by prolonged immunization of horses with B. typhosus toxins were used for the treatment of eighty-four patients with typhoid fever.

As controls, seven patients were treated intravenously with from 300 to 500 cc. of normal horse serum. Four died and three recovered. In only one case did the clinical course appear milder after the patient had received serum. Injection of large amounts of normal horse serum seemed to increase the severity of the disease and may have been a factor in the high death rate in the control series. In the series of patients treated with antitoxic serum, from 100 to 500 cc. was injected intravenously in divided doses over periods of from thirty-six to seventy-two hours. In forty of the eighty-four patients there was observed a striking influence on the toxemia, fever, bacteremia and duration of the disease. In thirteen patients there was a definite effect on the toxemia but no striking effect on the duration of the fever. In thirty-one there was no convincing influence of the serum on the course of the disease. Twelve of these received either inadequate amounts of serum, serum preparations of low antitoxic titer early in the work, treatment while moribund, or treatment within thirty-six hours of death from pneumonia. Of the eighty-four treated patients, seven died. Of these, one was moribund when serum was being given; one died of pneumonia within thirty-six hours after receiving the serum; two were given an early serum of low neutralizing potency; one died of pneumonia two weeks after the administration of serum; one died of perforation after a brilliant effect of the serum on the toxemia and fever. Only one patient died of toxemia (within twelve hours after the administration of serum).

THE CHEMOTROPIC ATTRACTION OF HUMAN LEUKOCYTES BY MICRO-ORGANISMS AND VARIOUS SUBSTANCES. MORTON MCCUTCHEON and HAROLD M. DIXON (by invitation), The University of Pennsylvania.

The chemotropic response of human polymorphonuclear leukocytes to different types of micro-organisms was evaluated through experiments in vitro. Under the microscope the net distance over which each cell approached a clump of bacteria was measured, and this distance was divided by the total length of the path of the cell. The resulting quotient was a measure of the chemotropic response, having as extreme values $+1$ if the cell moved directly toward the bacteria, and -1 if the cell moved directly away from them. With staphylococci, streptococci, pneumococci, typhoid and tubercle bacilli, Micrococcus tetragenus and certain yeasts, the mean quotients ranged only from $+0.73$ to $+0.86$, indicating approximately equal attraction under these conditions. With the yeast Torula histolytica the quotient was $+0.57$. Control leukocytes, wandering in fields free from known chemotropic influence, gave a value of $+0.07$. With substances other than bacteria a wide range of values was obtained: gelatin, 0.0; dried blood, $+0.18$; dried leukocytes, $+0.25$; starch paste, $+0.71$.

THE PRODUCTION OF ANTIBODIES WITHIN LYMPH NODES. PHILIP D. MCMASTER and STEPHEN S. HUDACK, The Rockefeller Institute for Medical Research, New York.

The Formation of Agglutinins Within Lymph Nodes.—When a suspension of killed Bacillus paratyphosus B was intradermally injected, on two successive days, in one ear only of a mouse, an extract from the node draining the lymphatic of that

ear a week later showed agglutinin in high concentration. Agglutinin was present, too, in the blood in lower concentration. None was found in the lymph node of the opposite side. The experiment was repeated in a large group of mice.

To evoke equivalent inflammatory reactions on both sides, a killed culture of B. paratyphosus B was injected intradermally into the right ear and Schick test toxin into the left. A week later agglutinin for B. paratyphosus B was found in the extract from the node on the side receiving the organisms, at a dilution of 1:240. It was present in the serum at a dilution of 1:60. None was found in the extract of the node of the opposite side.

In other experiments the same antigens were used but three hours later the ear inoculated with paratyphoid bacilli was amputated to avoid a possible drainage to the lymph node of antibody formed at the site of injection. Nine days later agglutinin was found in the extract of the lymph node on the side receiving the bacilli at a dilution of 1:120, and not in that of the lymph node on the other side. The serum gave a positive reaction at the dilution 1:60.

Killed cultures of Bacillus enteritidis and of Bacillus prodigiosus were injected intradermally into the ears of mice, the former on the right side, the latter on the left. From four to sixteen times as much agglutinin for B. enteritidis was found in the extracts from the nodes of the ears receiving this bacterial vaccine as in the extracts from the nodes of the other ears, and from three to ten times as much as in the serum. Similarly agglutinin for B. prodigiosus was found in far higher concentration in extracts of the nodes of the ears receiving these bacilli. Much less was present in the blood and still less or at most an equal concentration in the nodes on the other side. The lymph nodes on both sides were inflamed to about the same extent.

The longer the interval of time between the injection of the antigen and the examination for antibody up to three weeks, the greater was the concentration of the latter in both nodes and serum. With the passage of time the concentration in the serum equaled that in the nodes but did not exceed it.

A Comparison of the Formation of Agglutinin in the Lymph Nodes of Resistant and Susceptible Mice.—Highly resistant and susceptible mice of closely inbred strains, the reactions of which have been extensively studied by Webster and his collaborators, were obtained for the work through the kindness of these investigators.

Killed cultures of various agglutinin-forming bacteria were intradermally injected into the ears of groups of animals from these strains and a third mongrel strain, only one antigen being used for each experiment. After varying intervals of time the cervical lymph nodes draining the lymphatics of the ears were removed and the animals were bled for serum. The lymph nodes were extracted and the agglutinin titers of the extracts and of the serums compared in the various groups.

Almost invariably the concentration of agglutinin was slightly greater in the lymph nodes of the susceptible animals than in those of the resistant ones. So, too, in the case of the serum. The resistant group and the mongrel group formed approximately the same amount of agglutinin.

The mice of the strain manifesting a general lack of resistance seemed capable of forming slightly more specific agglutinin than those of the resistant strain.

GASTRIC SECRETION FOLLOWING IRRADIATION OF THE EXPOSED STOMACH AND THE UPPER ABDOMINAL VISCERA BY ROENTGEN RAYS. ALBERT M. SNELL and JESSE L. BOLLMAN, The Mayo Clinic.

Irradiation of the stomach is one of the methods that has been proposed for reducing gastric acidity in the treatment of peptic ulcer in man.

We have studied a series of normal dogs to determine the effect of irradiation on the gastric response to test meals and to stimulation with histamine. Irradiation of the exposed stomach was followed by a marked decrease in gastric acidity, and in some animals complete anacidity was produced temporarily. Irradiation of the upper portion of the abdomen also produced a temporary reduction in gastric acidity, but only occasionally was there a complete disappearance of free

hydrochloric acid. Irradiation of the extremities produced a definite but less extensive reduction in gastric acidity. After extensive irradiation of the stomach, sections of the gastric mucosa revealed no significant pathologic changes. We have been unable to produce permanent anacidity, in spite of the fact that irradiation was carried to the limits of tolerance. The dogs have been observed over a period of a year.

REESTABLISHMENT OF CORONARY CIRCULATION IN PROGRESSIVE CORONARY OCCLUSION. HAROLD F. ROBERTSON (introduced by ELLIOTT C. CUTLER), Peter Bent Brigham Hospital, Boston.

In coronary occlusion, the vitality of the heart muscle may possibly be maintained by a supply of blood from anastomosing coronary vessels, from rami telae adiposae, from vasa vasorum of the aorta, from backflow in thebesian vessels, or from backflow in the coronary sinus; or, when early panthoracopericardial adhesions are present, their vessels may help to supply the cardiac wall. Also, biochemical adjustments may sustain a heart when its normal vascular supply is diminished.

In a series of dogs an experiment was undertaken to determine which vessels might nourish the cardiac wall after the coronary vessels had been gradually occluded by ligature in stages. There being evidence that primary ligature of the cardiac veins gives optimum opportunity for a dilatation of coronary and thebesian vessels, this was first done in stages. A test of the theory that the heart muscle is supplied from a coronary sinus backflow was thus automatically omitted. The arteries were then tied in a series of operations, small vessels being ligated first. Finally, the vascular panthoracopericardial adhesions formed during the course of the experiment were separated, the animals usually dying as a result of fresh infarction of the cardiac wall following the separation of the adhesions.

As in many cases the heart tolerated coronary occlusion as long as these adhesions were preserved, it appears that their progressive development supplied the heart to a great extent. No increase was found in anastomoses of coronary vessels, rami telae adiposae or aortic vasa vasorum; in some cases thebesian vessels were found dilated. The possible ability of the former channels to proliferate or of dilated thebesian vessels to function in compensation for a progressive coronary occlusion was not evident, but may have been taken over by the unavoidable formation of the vascular adhesions.

Slides of hearts in gross are presented to show vascular adhesions, and sections, to show dilated myocardial vessels.

STUDIES OF EXPERIMENTAL CORONARY OCCLUSION. ROBERT TENNANT JR. (introduced by RAYMOND HUSSEY), Yale University.

After ligation of the anterior descending branch of the left coronary artery in dogs the lactic acid content of heart muscle from the affected zone was increased from 100 to 200 per cent over that of control muscle from the right ventricle, irrespective of the duration of the period of ligation up to twenty-four hours. The glycogen content of muscle from similar zones was decreased in every instance. In a second group, in which reestablishment of circulation was effected by removal of the ligatures after intervals varying from a half hour to eight hours, and the animals were sacrificed two hours later, the lactic acid content of muscle from the involved zone remained lower than that of muscle from the control zone, as did also the glycogen content. No histologic changes were observed in the myocardium of any of the animals in the first group up to twelve hours. On the other hand, in the second group, in which the circulation was reestablished, there were extensive hemorrhage, edema, polymorphonuclear leukocytic exudate and slight necrosis throughout the involved zone. In both groups the electrocardiographic changes consisted of T-wave alterations, ectopic premature ventricular contractions, ventricular tachycardia and ventricular fibrillation. The premature contractions appeared within from six to eight hours after the ligation of the vessels; in the second group they appeared earlier.

THE NATURE OF THE INTIMA AS A TISSUE. ELIZABETH M. RAMSEY and DAVID W. GAISER (introduced by RAYMOND HUSSEY), Yale University.

A strain of Streptococcus viridans known to be pathogenic for rabbits was injected into the rabbit carotid artery between two ligatures. The observations on a series of rabbits were as follows: Six hours after the injection a polymorphonuclear leukocytic exudate was seen in the adventitia of the vessel. At twelve hours the exudate had reached the midportion of the media. At from twelve to twenty-four hours the exudate was present throughout the wall of the vessel and particularly densely accumulated just outside the internal elastic membrane. Between twenty-four and seventy-two hours the exudate was seen also in the lumen of the vessel, and the elastic fibers of the media were separated by dense accumulations of leukocytes. In the later stages, necrosis of fixed tissues became apparent associated with the exudate. At all periods there was cellular infiltration in the connective tissue surrounding the vessel. As the exudate in the wall of the vessel became more widespread and more dense there was also an increase in the amount of exudate in the periarterial tissues, but the infiltration was always most marked in the wall of the vessel. Bacterial stains showed large numbers of organisms in the lumen of the vessel, but none were seen elsewhere. Blood vessels in control animals subjected only to the operative procedure and to double ligation for equal periods of time showed no detectable changes in the wall of the vessel in stained preparations and only slight hemorrhage and minimal cellular infiltration in the periadventitial tissues.

These observations seem to indicate that diffusible substances associated with the streptococcus penetrate the wall of the vessel from within and call forth a leukocytic response. The cells of the exudate apparently issue from the periadventitial vessels and progressively infiltrate the whole wall, finally filling the lumen.

EARLY INCIDENCE OF SPONTANEOUS MEDIAL DEGENERATION ("ARTERIOSCLEROSIS") IN THE AORTA OF THE RABBIT. H. D. KESTEN, Columbia University.

This article will appear in full in a later issue of the ARCHIVES.

FUNCTIONAL HYPERTROPHY OF THE KIDNEY. J. L. BOLLMAN and F. C. MANN, The Mayo Foundation.

This article will appear in full in a later issue of the ARCHIVES.

THE TITRATION OF PROTHROMBIN IN CERTAIN PLASMAS. E. D. WARNER (by invitation), K. M. BRINKHOUS (by invitation) and H. P. SMITH, State University of Iowa.

If one adds to plasma an optimum amount of calcium plus an excess of thromboplastin, the prothrombin present will be converted completely into thrombin. By suitable procedures one can determine the relative amount of thrombin present and hence the relative amount of prothrombin in the original plasma. Certain pitfalls in this titration of prothrombin are discussed. An excess of antithrombin, especially, interferes with the estimation of thrombin. The antithrombin can be eliminated by ammonium sulphate fractionation.

Experiments on dogs show that in from twenty to thirty hours after chloroform poisoning the plasma prothrombin has fallen to less than 5 per cent of normal. Recovery occurs within a week. The fall in plasma fibrin is less extreme. The rise to normal is parallel to the rise in prothrombin.

Peptone plasma, after being freed from its antithrombin, can be shown to have a normal content of prothrombin.

THE UTILIZATION OF PLASMA PROTEIN (DOG AND HORSE) AND HEMOGLOBIN GIVEN BY VEIN. WESLEY T. POMMERENKE (introduced by GEORGE H. WHIPPLE), The University of Rochester, Rochester, N. Y.

Evidence is presented showing that the level of the circulating plasma protein in dogs can be raised to a value about 50 per cent above the normal by intravenous injection of heparinized dog plasma. This seems to produce no untoward results. When sugar is also given by mouth, the animals can be maintained practically in nitrogen equilibrium. The surplus protein is removed from the circulation and does not escape in the urine. This seems to point to a utilization of the administered protein in the bodily economy.

When horse plasma or dog hemoglobin is given intravenously under identical conditions, there is little evidence that these proteins are utilized to spare body protein.

FORMATION OF INTERCELLULAR SUBSTANCE BY ADMINISTRATION OF ASCORBIC ACID (VITAMIN C) IN EXPERIMENTAL SCORBUTUS. S. BURT WOLBACH, VALY MENKIN and MIRIAM F. MENKIN (by invitation), Harvard Medical School.

Scurvy is characterized by a cessation in the normal formation of intercellular substance on the part of supporting tissues. This was established by the earlier studies of Wolbach and Howe (1926). The immediate effect of vitamin C administered in the form of orange juice to guinea-pigs was a prompt reparative response. This process was clearly demonstrated in several ways, notably by the renewed formation of dentin in the incisors of guinea-pigs, the deposition of a homogeneous matrix by the periosteal layer of cells, and finally, the formation of osteoid and osseous trabeculae in the *Gerüstmark* at the costochondral junctions. More recently Wolbach demonstrated that the deposition of collagen in the organization of blood clots in the state of absolute scurvy is referable to the administration of vitamin C, and that it doubtless represents the product of fibroblastic secretory activity.

In an endeavor to determine whether these histologic effects were due to vitamin C per se we isolated this vitamin in crystalline form (ascorbic acid) from lemon juice and administered it to scorbutic guinea-pigs. The ascorbic acid was administered orally or parenterally in a dosage of from 3 to 5 mg. a day. The animals were killed at from three to fifteen days. The reparative reactions were studied in microscopic sections of both the incisor teeth and the costochondral junctions. Typical responses, as previously obtained with orange juice and as stated in the foregoing paragraph, were observed. In an animal which had received four parenteral injections of ascorbic acid the deposition of osteoid matrix in the *Gerüstmark* was particularly marked. Areas of newly formed cartilage were frequently found within the recently deposited collagen matrix at the costochondral junction. In a guinea-pig killed at about two weeks the newly formed osteoid trabeculae at the *Gerüstmark* were found ossified with evidence of progressive bone resorption. This study clearly demonstrates that the cessation in the deposition of intercellular substance in experimental scurvy is due to the lack of ascorbic acid, a relatively simple chemical substance, having as its empirical formula $C_6H_8O_6$.

HETEROTOPIC FORMATION OF TEETH. C. B. HUGGINS, H. R. MCCARROLL (by invitation) and A. A. DAHLBERG (by invitation), The University of Chicago.

Using a method that we have already described (Huggins, C. B.; McCarroll, H. R., and Dahlberg, A. A.: *Proc. Soc. Exper. Biol. & Med.* **31**:525, 1934), we found it possible to induce the formation of enamel, dentin and cementum in the abdominal fascia of puppies, by autogenous transplantation of the soft tissues of the developing germ of the permanent canine tooth.

The results may be summarized as follows: Transplantation of the ameloblast layer with its subjacent capillary bed and connective tissue, in a "normal" relationship to the pulp, leads to new formation of enamel and dentin, with persistence of the cylindric character of the ameloblasts. But if the approximately normal

relationship of these layers is not maintained, the cylindric form of the ameloblasts does not persist, the epithelium survives in a stratified squamous form, and no enamel is produced. Thus transplantation of the ameloblast layer alone leads to islands and cords of stratified squamous epithelium, at times with keratohyaline pear formation, but without new formation of enamel.

Transplantation of the odontoblast layer of the pulp leads consistently to dentin formation in eleven or more days.

Hertwig's sheath was successfully transplanted in a number of instances.

RELATION OF VIOSTEROL AND PARATHORMONE IN PUPPIES ON CONTROLLED DIETS. G. R. SHARPLESS (introduced by F. W. HARTMAN), Henry Ford Hospital, Detroit.

Three groups of growing pups were fed modified Cowgill diets (*J. Biol. Chem.* **56**:725, 1923) and treated with parathormone. Group 1 received a diet low in vitamin D with a calcium content of 0.33 per cent and a ratio of calcium to phosphorus of 1:1.8; group 2 received the same diet and two drops of viosterol (2,500 D) per dog daily; group 3 received the same diet as group 1 except that no calcium was added.

Group 1 tolerated parathormone well, receiving 2,200 units of parathormone in a period of four months and gaining in weight from 3.32 to 6.02 kilograms. Group 2 did not acquire "immunity" to parathormone, received an average of only 630 units and gained 0.5 kilogram in weight in three and one-half months. Group 3 quickly acquired "immunity" to parathormone, received 2,150 units in three months and gained weight as fast as the controls on the same diet.

At autopsy, group 1 showed absence of subcutaneous and omental fat, but no calcium deposits were visible in gross. Group 2 showed metastatic calcification in the stomach, heart, kidneys and thyroid gland. An x-ray picture showed suggestive cyst formation in the femur of one dog in group 2, but no change in either of the other groups. Group 3 appeared perfectly normal and well nourished. In none of the groups were the bones deformed or extremely soft.

PARATHYREOTROPIC ACTION OF ANTERIOR LOBE OF THE PITUITARY GLAND; HISTOLOGIC EVIDENCE IN THE RABBIT. SAUL HERTZ and ALFRED KRANES (introduced by JAMES H. MEANS), Massachusetts General Hospital, Boston.

On the basis of a preliminary observation that emulsions of fresh anterior lobe of the pituitary gland gave rise to an enlargement and increased vascularity of the parathyroid glands of the rabbit, a more complete histologic study was undertaken. In a series of seven experiments the parathyroid glands of forty-eight rabbits subjected to injections of anterior lobe extract and of pregnancy urine as well as of several proprietary preparations from these sources were grossly and microscopically compared with those of seventeen reference control animals. The latter either received no injections or were given injections of heated pituitary, normal urine or emulsion of fresh brain substance.

The animals which had been treated with anterior lobe preparations and extracts of pregnancy urine exhibited parathyroid glands which were grossly larger and more vascular than those of the control group. They showed histologic changes consistent with hypertrophy and hyperplasia. The criteria for the latter described by Erdheim in reference to the rat were applied.

THE PROSTATE AND SEMINAL VESICLES AS QUANTITATIVE INDICATORS OF THE MALE SEX HORMONE. ROBERT A. MOORE, Cornell University.

The male sex hormone content of any extract may be measured by the increase in the size of the comb in the capon or by the height of the prostatic and vesicular epithelium of the castrated rat. In confirmation of the investigation carried out by Hansen, I have found that the height of the epithelium of the adult male rat, castrated and treated by injection for ten days and killed on the eleventh day, shows a direct relationship to the amount of hormone injected.

CORRELATION OF ANTEMORTEM AND POSTMORTEM BACTERIOLOGY. CASPAR G. BURN (introduced by RAYMOND HUSSEY), Yale University.

In a bacteriologic study of eighty-three cases in which blood cultures were made both before and after death, agreement was found in fifty and disagreement in thirty-three. Among the cases in which there was disagreement there were five in which contamination of the blood cultures was the result of technical difficulties in the collection of the material. Among the remaining twenty-eight there were nineteen in which the last clinical culture was taken three days or longer before death, and nine in which the interval before death was less than forty-eight hours. In contrast, among instances in which the antemortem and postmortem cultures were in agreement there were 31 in which the clinical cultures were taken within forty-eight hours of death. Bacteriologic studies of the organs in fifty-seven of the eighty-three cases demonstrated bacteria other than the kind isolated from the blood stream. Postmortem blood cultures agree in a significantly large number of instances provided the antemortem cultures are obtained shortly before death. Organs at necropsies contain pathogenic bacteria other than the kinds isolated from the blood stream.

THE INFLUENCE OF COPPER IN THE DIET ON THE RESISTANCE OF ALBINO RATS TO TRYPANOSOMAL INFECTIONS. DAVID PERLA (introduced by DAVID MARINE), Montefiore Hospital, New York.

In previous work it was found that the addition of small amounts of copper to an adequate diet for rats protected a large percentage of the rats from Bartonella muris anemia following splenectomy. Further studies were undertaken to determine whether copper added to the diet would raise the natural resistance of adult albino rats to trypanosomal infections. The addition of copper (0.1 mg. per rat per day) or iron (1 mg. per day) or both to an adequate diet during a period of ten days prior to an induced infection with Trypanosoma Lewisi raised the natural resistance of the rats to this infection. The infection was completely aborted in almost 50 per cent. Lead salts added to the diet had no beneficial effect.

The addition of copper in amounts of from 0.2 to 0.4 mg. per rat per day to an adequate diet during a period of ten days prior to an induced infection with Trypanosoma equiperdum, a fatal infection in rats, strikingly raised the natural resistance of the rats to this disease. Adult albino rats infected with overwhelming doses of T. equiperdum, if previously fed copper, survived slightly longer than the controls. Adult albino rats fed a diet supplemented with 0.4 mg. of copper per rat per day during a period of ten days prior to the injection of 10,000 trypanosomes (T. equiperdum) per rat developed only an abortive infection and 60 per cent recovered, whereas all the controls died. When the rats were infected with 2,000 trypanosomes per animal, all the copper-fed rats developed abortive infections and recovered, but 62 per cent of the controls died.

It is concluded that the species susceptibility of rats to trypanosomal infections may be markedly altered by supplementing their diet with copper prior to infection.

CELLULAR CHANGES IN THE SPLEEN IN PERNICIOUS ANEMIA. RAPHAEL ISAACS, University of Michigan.

In films of serum suspensions of the spleens of patients with pernicious anemia there is a relative and an absolute increase in the number of elongated bipolar cells with oval nuclei (9 by 6 microns) having the characteristics of "connective tissue cells." The isolated cells have two long cytoplasmic processes, occasionally with bifurcated ends. These are from 90 to 105 or more microns in length and average 1.5 microns in width. A similar increase in the number of these cells is noted in fetal spleens and, to a lesser degree, in the spleens of patients who have had considerable irradiation with roentgen rays. The increase in the number of these cells appears to be characteristic of pernicious anemia, the cells constituting from 13 to 25 per cent of all nucleated cells as compared with less than 6 per cent in other conditions. Data on the number of these cells in relapse and in remission of pernicious anemia and in certain other diseases are given.

THE NATURE OF THE RESPONSE TO INFECTION IN DYSCRASIA OF BONE MARROW. FRANK H. BETHELL (introduced by CYRUS C. STURGIS), University of Michigan.

During the course of an infection alterations in the maturity of the neutrophils as evidenced by their nuclear form are dependent on the severity of the infection and the functional state of the bone marrow prior to the acute illness. Cytoplasmic changes in the neutrophils and in particular the appearance of basophilic or "toxic" granules in Wright-stained preparations are related solely to the infective process, being quite independent of the nature of the bone marrow response. That such a modification of the staining characteristics of the neutrophil cytoplasm is not a merely local phenomenon dependent on exposure to a site of inflammation is attested by the evidence gained from successive estimations of the total neutrophil count, the nuclear shift and the incidence of basophilic granulation in the immature and adult classes. During the period of exacerbation basophilic granulation is increasingly prevalent in the immature neutrophils most recently released into the circulation.

In cases of abnormal bone marrow response such studies provide an insight into the nature of the myeloid dysfunction. The examples of atypical response to infection which I shall report comprise pernicious anemia in an aregenerative phase and at the beginning of a therapeutically induced remission, postarsphenamine aplastic anemia, agranulocytic angina and aleukemic lymphatic leukemia.

FUNDAMENTAL BONE MARROW REACTIONS: II. THE EFFECT OF SMALL DOSES OF X-RAYS AND RADIUM ON MYELOPOIESIS IN THE PIGEON AND RABBIT. CHARLES A. DOAN and LOWELL A. ERF (by invitation), Ohio State University.

Nakahara in Murphy's laboratory believed that he was able with very small doses of x-rays to produce a primary stimulation of cells in the germinal centers of lymph nodes as reflected by an increase in the number of mitotic figures. However, he noted no change in the bone marrow elements.

In a series of experiments in rabbits, Sabin, Doan and Forkner, using monthly injections of radium chloride and mesothorium, 5 and 7 micrograms respectively, produced definite pathologic changes but, despite extensive foci of necrosis and fibrosis in the bone marrow, granulopoiesis continued to maintain the neutrophils at an approximately normal level through the experimental period of from nine to sixteen months. No absolute neutrophilic leukocytosis occurred as a result of the injection of these radioactive materials.

In pigeons, in which myelopoiesis and erythropoiesis are sharply separated and aplasia and hyperplasia coexist in different areas, we have used doses of x-rays of high voltage ranging from 19 roentgens (1/40 skin erythema dose) to 790 roentgens (1 skin erythema dose), both singly and in series, and have in no instance found any increase in mitosis of myeloid elements in the absence of definite myelocytic destruction. Biopsies were made in each instance before irradiation, and any change in the gross or microscopic evidence of myelopoiesis was always found to be directly proportional to the extent of specific myelocytic nuclear degeneration and karyolysis. No absolute increase in circulating granulocytes resulted in any of these animals.

We therefore conclude that it is probably impossible to secure primary myelopoietic stimulation by the use of even the smallest, so-called, therapeutic doses of x-rays.

EFFECT OF THE HOST CONSTITUTION ON THE LYMPHOID CELLS OF TRANSMISSIBLE MOUSE LEUKEMIA. J. S. POTTER and JOSEPH VICTOR (introduced by JAMES W. JOBLING), The Carnegie Institution of Washington and Columbia University.

Two lines (I and M-Liver) of cells of transmissible lymphatic leukemia which have been carried in genetically homogeneous mice of strain C58 yield similar gross and microscopic pictures and kill hosts within similar periods of time. When

either of these lines is carried through mice of the highly inbred Storrs-Little strain, the picture presented by the infiltration is the same for the first seventy-two hours as that found in strain C58. After this period the lesions may increase in size and number, eventually causing death of the host, or they may regress, terminating in recovery of the host.

The cells of both these lines show a reduced mean number of mitochondria when growing in hosts of the Storrs-Little strain. In line M-Liver the mode as well as the mean is changed; in line I only the mean is changed. Cell size and other morphologic traits remain the same in hosts of each strain.

The changes in the number of mitochondria are coincident with changes in the glycolytic rate in these transmission lines, suggesting a direct relationship. When line cells are returned to hosts of strain C58 all functions return to the level observed before the passage through the Storrs-Little strain.

THE METABOLIC EFFECT OF THE HOST CONSTITUTION ON THE LYMPHOID CELLS OF TRANSMISSIBLE MOUSE LEUKEMIA. JOSEPH VICTOR and JAMES S. POTTER (introduced by JAMES W. JOBLING), Columbia University and The Carnegie Institution of Washington.

The coefficients of variability for repeated determinations of the oxygen consumption and the aerobic and anaerobic glycolysis of a given line of leukemic lymphocytes carried by host mice of strain C58 vary from 3 to 15 per cent. This variability, smaller than any reported in the literature for transplantable tumor, may be attributed in part to the genetic uniformity of mice of strain C58. Normal lymphocytes of this strain do not differ metabolically from those of another genetically uniform strain of mice, Storrs-Little. When leukemic cells of line I were transferred from strain C58 to hosts of the Storrs-Little strain, the oxygen consumption increased, and the aerobic and anaerobic glycolysis diminished; leukemic cells of line M-liver gave a similar reduction in glycolysis, but no change in oxygen consumption. When these two lines of cells were transferred from a Storrs-Little donor to hosts of strain C58, the metabolic rates returned to their original levels. These results indicate that the genetic constitution of the host affects the metabolism of leukemic cells, and that different lines of cells may be differentially affected by the same host. Furthermore, the metabolic effect of a host on leukemic cells is temporary and present only when the cells are in that host.

CHANGES IN VIRULENCE AND SIZE IN LINES OF LEUKEMIC LYMPHOCYTES CARRIED BY MICE. MAURICE N. RICHTER and E. C. MACDOWELL (by invitation), Columbia University and The Carnegie Institution of Washington.

Changes in the interval between inoculation and death are frequently observed during the course of successive transfers of a line of leukemic lymphocytes. When uniform technic and genetically and ontogenetically homogeneous hosts are used, these changes indicate changes in the cell line. The changes usually but not always shorten this interval. They may occur at any time and at any rate from abrupt changes within a single transfer to very slow ones extending over a long series of transfers. Stable periods with constant averages and degrees of variability occur at any time after the first three transfers.

Cytologic studies have been made on specially prepared material covering the periods of more rapid change in the aforementioned interval. The observations include the cell size, the rate of division and the ratio of the nucleolus to the nucleus. In periods when this interval is stable cytologic traits are stable. Cytologic changes are found to occur during periods of change in this interval and indeed may be detected in advance of the latter change. Cell traits may alter gradually over a series of transfers that include a sudden break in the length of this interval. Cell changes are antecedent to changes in this interval, but threshold phenomena in certain cases may lead to abrupt changes in the interval.

A Neoplastic Disease with Nuclear Inclusions Occurring in the Leopard Frog. Balduin Lucké, The University of Pennsylvania.

In about 2 per cent of leopard frogs (Rana pipiens) there has been observed a neoplastic disease which manifests itself by the occurrence of unencapsulated whitish tumors in the kidneys. In the majority of the cases these tumors have the histologic appearance of adenocarcinoma.

Two hundred and four frogs with such tumors have been studied. The disease occurs in the two sexes and in the two kidneys with approximately the same frequency. The tumors are commonly multiple and in about one half of the cases occur bilaterally. They vary in size from a minute nodule to a mass several times larger than a normal kidney. While the new growths are locally destructive and infiltrative, they rarely metastasize.

In the majority of the tumors there are present characteristic nuclear changes, namely, a condensation of chromatin at the periphery of the nucleus and very numerous and prominent acidophilic inclusion bodies lying within nuclei which otherwise appear almost empty. The inclusions are of the same general appearance as those of herpes, chickenpox and certain other virus diseases. They occur only in the epithelial cells of the tumors. Such inclusion bodies are currently regarded as presumptive evidence of the activity of a virus; however, proof of the existence of a virus and of its relation to the tumor cannot be furnished.

Material from twenty-four different tumors has been transplanted by various methods into several regions of four hundred and seventy-eight frogs. In a relatively small number of animals the transplants have survived for upward of three months and exhibited some evidence of growth, but no massive tumors have developed at the sites of inoculation. However, in a number of the animals large tumors did arise in the kidneys; this may or may not be evidence of transmission. It should be pointed out that if the causal agent of this neoplastic disease is a virus the virus would be expected to localize and produce, under experimental conditions, lesions in that tissue (in this case, the kidney) in which the spontaneous growths occur. Further experiments are necessary to establish the nature of the tumors. They appear to be promising material for various studies on new growths.

The Proliferative Reaction of Guinea-Pig Skin to Sulphydryl and Its Relation to Neoplasia. Stanley P. Reimann and Ethel Rahe Hankele (by invitation), The Lankenau Hospital Research Institute, Philadelphia.

This article will appear in full in a later issue of the Archives.

An Apparently Hereditary Form of Splenomegaly with Cirrhosis of the Liver in the Rabbit. Harry S. N. Greene, Paul D. Rosahn and C. K. Hu (introduced by W. H. Brown), The Rockefeller Institute for Medical Research, New York.

A pathologic complex consisting of diffuse fibrosis of the spleen and portal cirrhosis of the liver has been found in a line of inbred rabbits with a frequency suggesting that it is hereditary. The condition bears a marked resemblance to the splenic anemia of man and is being studied with a view to determining its relationship to this symptom complex and its pathogenesis.

The Effect of Oxygen in Prevention of Hepatic Necrosis Produced by Volatile Anesthetics. S. Goldschmidt, I. S. Ravdin and B. Lucké, The University of Pennsylvania.

A comparison has been made of the relative incidence of hepatic necrosis in dogs anesthetized in a semiclosed system when the anesthetic was volatilized with air, and when it was volatilized with oxygen. The data show that the use of oxygen with either divinyl ether or chloroform is a potent factor in the reduction of postanesthetic necrosis of the liver.

When divinyl ether was used as the anesthetic by any method, necrosis of the liver was not observed with any degree of regularity until the anesthesia had been maintained for a period of three hours. The incidence of postanesthetic necrosis after three hours of anesthesia in a semiclosed system was nearly twice as high when the anesthetic was volatilized with air as with oxygen.

Chloroform anesthesia, on the other hand, resulted in a high incidence of hepatic necroses in dogs after one hour of anesthesia. The incidence of necrosis of the liver following one hour of chloroform anesthesia in a semiclosed system was approximately ten times as great when the anesthetic was volatilized with air as with oxygen.

Hepatic degeneration has been produced in the dog following three hours of ether anesthesia when the anesthetic was volatilized with less than the atmospheric pressure of oxygen, i. e., oxygen 15 per cent and nitrogen 85 per cent.

Data demonstrating the efficacy of oxygen during anesthesia will be presented, and the general implications of our findings will be discussed.

SERUM PHOSPHATASE IN EXPERIMENTAL INSUFFICIENCY OF THE LIVER. F. W. HARTMAN and VICTOR SCHELLING (by invitation), Henry Ford Hospital, Detroit.

In the course of experimental insufficiency of the liver produced with a modified Eck fistula and x-rays of high voltage, renal stones as noted by other observers were found in about 50 per cent of the animals that lived more than six months after operation. This observation led us to determine the phosphatase in normal animals and in animals with hepatic insufficiency produced by Eck's fistula, Eck's fistula plus irradiation with x-rays of high voltage and carbon tetrachloride. All of these groups showed a marked increase in serum phosphatase ranging from five to ten times the normal. Further there is a close parallel between the increase in phosphatase and the function of the liver as determined by cholesterol partition, bilirubin and bromsulphalein.

INTOXICATIONS OF DOGS WITH BILIARY FISTULA. WILLIAM B. HAWKINS, The University of Rochester, Rochester, N. Y.

Three types of dogs with biliary fistula have been studied over long periods with observations as to the different states of intoxication that develop following deprivation of the intestinal tract of bile.

Such animals are subject to gastro-intestinal upsets if not fed a suitable diet.

In dogs with open infected fistula and obstructed infected closed fistula the bones are progressively decalcified, with resulting multiple fractures. Liver added to the diet prevents or delays this change. Dogs with renal fistula may live for long periods deprived of bile without this decalcification.

Fistulous dogs deprived of bile show purpuric tendencies with hemorrhage from the gastro-intestinal tract or trivial wounds. Their blood reveals normal fibrinogen and normal platelets, and the blood will clot in a test tube after some delay. Serum added to recalcified plasma causes the formation of the clot in normal time. Whole bile or bile salts given by mouth apparently restore conditions to normal with a disappearance of the bleeding tendency.

Fifty cubic centimeters of whole dog or ox bile by mouth will maintain a fistulous dog in a healthy condition for long periods of time.

Combination biliary and Eck fistulas are not well tolerated by dogs.

THE OVERPRODUCTION OF BILIARY PIGMENT IN SPLENECTOMIZED DOGS WITH BILIARY FISTULA. RALPH E. KNUTTI, The University of Rochester, Rochester, N. Y.

In splenectomized dogs with biliary fistula periods of marked production of bilirubin occur. Associated with such periods is a massive destruction of red blood cells. The amount of biliary pigment produced at these times is greatly in excess

of the amount that can be accounted for by the bilirubin equivalent of destroyed hemoglobin, although there are very rapid destruction and rebuilding of this substance. The occurrence of these periods has been found to be related to the presence in the red blood cells of bodies indistinguishable from those of Bartonella canis. Modification and disappearance of such periods have been brought about by the oral administration of an extract of spleen. An attempt to explain the disparity of the ratio of bilirubin to hemoglobin has been made.

BILIARY CHOLESTEROL: FLUCTUATIONS DUE TO DIETARY FACTORS, BILIARY SALT, INJURY TO THE LIVER and HEMOLYSIS. ANGUS WRIGHT (introduced by GEORGE H. WHIPPLE), The University of Rochester, Rochester, N. Y.

Observations were made on dogs with sterile closed biliary fistulas. The method used in determining the values of biliary cholesterol was quantitatively accurate. The effect of various factors on the excretion of biliary cholesterol was determined after control levels had been established on a basal diet. Under uniform dietary conditions dogs eliminate fairly constant amounts of biliary cholesterol. Diets rich in cholesterol raise the cholesterol output in the bile. Biliary salt alone raises the biliary cholesterol as much as or more than a diet rich in cholesterol. Biliary salt plus a cholesterol-rich diet gives the maximal output of biliary cholesterol. Hepatic injury decreases the elimination of both cholesterol and biliary salt in the bile. Destruction of red blood cells fails to increase the output of biliary cholesterol.

COMPARISON OF THE RESULTS OF THE WASSERMANN TEST IN TWO LABORATORIES. DAVID L. BELDING, The Massachusetts Memorial Hospitals, Boston.

A statistical study of the Wassermann test on 10,000 duplicate samples of serums of known reaction strength from treated syphilitic patients was made in two laboratories. Of the serums 30 per cent gave reactions of less than 1 unit; 20 per cent, above 4 units, and 50 per cent, between 1 and 4 units.

If these serums had been tested in one laboratory, 17 per cent of the positive reactions would have been missed. Lack of agreement was almost entirely confined to the borderline group of serums with reactions of from 1 to 4 units, in which there was 35 per cent disagreement. The daily variation in laboratory technic was the chief cause of this disagreement. The daily variations from an arbitrary mean of 100 gave standard deviations of 34.9 and 23.7, respectively, for the two laboratories.

THE EFFECT OF IODINE AND VITAMIN B_1 ON THE THYROID, SUPRARENAL AND HYPOPHYSEAL GLANDS OF THE RAT. M. D. CARPENTER and G. R. SHARPLESS (introduced by F. W. HARTMAN), Henry Ford Hospital, Detroit.

A study has been made of the effect of iodine and of vitamin B_1 on the weight of the thyroid, the suprarenal and the hypophyseal glands, the structure of the thyroid gland and the iodine content of this gland. The rat was used, and studies were made at the end of 41, 82 and 123 days of the experimental diet.

The results calculated as weight of gland per hundred grams of body weight show a definite enlargement of all three glands during deficiency of vitamin B_1. The percental enlargement is much greater for the thyroid than for the suprarenal or the hypophyseal gland. There seems to be no consistent correlation between the size of either the suprarenal or the hypophyseal gland and the iodine intake, but the thyroid gland is definitely smaller in the animals with the higher iodine intake.

Iodine determinations on the thyroid gland show that an increased iodine intake increases the iodine content of this gland, but the percental iodine content of the gland does not seem to be closely correlated with the size of the gland.

Microscopically, definite extensive hyperplasia of the thyroid gland was evident only in those rats receiving a diet low in iodine and optimal in vitamin B_1. There is some evidence of accumulation of dense colloid in those receiving iodine during deficiency of vitamin B_1.

THE IODINE CONTENT OF THE BLOOD IN DISEASES OTHER THAN THOSE OF THE THYROID GLAND. GEORGE M. CURTIS, Ohio State University.

The majority of hospital patients with diseases other than those of the thyroid gland present a normal content of iodine in the blood. Among the diseases investigated were Hodgkin's disease, tuberculosis, cancer, fractures, chronic osteomyelitis and functional disorders. Likewise, the majority of ambulatory patients with such diseases present a normal content of iodine in the blood. There are, however, certain exceptions. In acute severe infections—for example, septicemia—the level of the blood iodine is elevated. It is likewise elevated in lymphatic leukemia. It rises immediately following a major surgical operation for disease other than that of the thyroid gland. It is elevated in certain forms of heart disease and in hypertension. Since the level of the blood iodine is a measure of the activity of the thyroid gland, it is possible to use it in evaluating the thyroid component in diseases other than those of the thyroid gland.

CHANGES IN THE CENTRAL NERVOUS SYSTEM RESULTING FROM CONVULSIONS DUE TO HYPERINSULINISM. DAVID M. GRAYZEL (introduced by RAYMOND HUSSEY), Yale University.

Repeated convulsions for varying periods of time were induced artificially by the production of hyperinsulinism. The convulsions were produced by the intravenous injection of insulin into rabbits that previously had been made to fast for eighteen hours. The dose of insulin required to produce an attack varied considerably (from 2 to 12 units) for different animals. The attacks occurred within from two to six hours after the injection of the insulin. The convulsions were allowed to continue for periods of time ranging from three minutes to several hours. They were then terminated by the intravenous injection of 10 cc. of 50 per cent dextrose. Whenever possible the experiments were conducted over a period of three months. At the close of the experiments the animals were killed with ether and examined. Blocks of tissue were taken from various parts of the brain, fixed in 95 per cent alcohol and stained with toluidine blue by the Nissl method. The lesions were arbitrarily graded from negative to 4 plus depending on the severity.

From the results it was apparent that animals which had not been convulsed, or only slightly, showed either minimal or no cerebral changes regardless of the number of injections of insulin that they had received. On the other hand, even one convulsion, if prolonged and severe enough, produced definite lesions in the central nervous system, of the necrobiotic type. Furthermore, the more prolonged and the more severe the convulsions, the more extensive were the lesions.

GENETIC VARIATIONS IN THE PERIOD OF GESTATION OF THE RABBIT. PAUL D. ROSAHN, HARRY S. N. GREENE and C. K. HU (introduced by W. H. BROWN), The Rockefeller Institute for Medical Research, New York.

During the past few years this laboratory has maintained a colony comprising approximately one thousand five hundred standard-bred and hybrid rabbits. The data accumulated include accurate records of the date of mating and the date of birth of the offspring of all animals raised in the colony. This information has been submitted to a statistical analysis, with the general purpose of determining the period of gestation of the rabbit, and the particular purpose of determining whether the breed of rabbit has any influence on the duration of pregnancy. The mean gestation period has been calculated for several standard-bred lines, and it has been found that statistically significant differences exist between different breeds. Moreover, the variation between breeds was significantly greater than the variation within the breeds. These findings indicate that the genetic factors which are responsible for differences in breed significantly influence the period of gestation of the rabbit.

HEREDITARY HYDROCEPHALUS IN THE RABBIT. HARRY S. N. GREENE, C. K. HU and PAUL D. ROSAHN (introduced by L. PEARCE), The Rockefeller Institute for Medical Research, New York.

The occurrence of hydrocephalus in certain hybrid lines of the rabbits in our breeding colony has long attracted our attention. The condition is manifested early in life in the young of male and female transmitters.

It appears that this abnormality has more than one form. The primary variations responsible for the difference in form have not been determined. In some instances the hydrocephalus is internal and in other cases it is external. Furthermore, it may or may not be associated with deficient or retarded calcification of the membranous bones of the skull.

Most of the animals manifesting the abnormality die early in life. However, recent experiments indicate that a therapeutic measure for hastening the calcification of the calvarium is capable of checking the progress of the hydrocephalus, and life may be prolonged and possibly saved. Further investigations are in progress.

It is possible that such a therapeutic measure for hastening calcification of the calvarium, when carried out early enough, may prove to be of practical use in certain cases of hydrocephalus in man.

METASTASIS OF A SQUAMOUS CELL CARCINOMA FROM THE WRIST TO THE AXILLA WITHOUT DEMONSTRABLE INTERVENING GROWTH. ESMOND R. LONG, The Henry Phipps Institute, Philadelphia.

Since opinion is divided as to whether lymphatic spread from a primary carcinoma to distant secondary lymph nodes occurs by lymphatic embolism or by continuous lymphatic growth a study was made of the entire amputated arm from a man with a carcinoma of the wrist.

The patient, 59 years of age, entered the Billings Hospital of the University of Chicago with a squamous cell carcinoma of the right wrist. Enlarged glands were palpable in the right axilla. The limb was amputated in the middle third of the upper part of the arm, and the lymph nodes in the axilla were removed *en masse* by block resection. Several of these contained carcinomatous nodules. The patient made an uneventful recovery. Two years later he was well, with no evidence of recurrence.

Sections were taken from serial blocks from the wrist to the upper end of the lower third of the amputated arm. The arm was first fixed in Zenker's solution by injection through the main arteries, and sawed into twenty-seven consecutive blocks from 1 to 1.5 cm. thick. The bones were then removed and the blocks embedded in pyroxylin (celloidin). Several representative sections from each block were stained with hematoxylin and eosin. Blocks 1 and 2, from the wrist, contained carcinoma with definite extension into the lymphatics. In block 3 a few cells thought to be from the carcinoma were seen. Considerable granulation tissue was present at the site corresponding to the carcinoma in the previous blocks. In blocks 4 and 5 traces of granulation tissue persisted, but no carcinoma cells were seen. In block 7, the first block with large muscle trunks, a scattered lymphangitis was discovered, several lymphatics being plugged with polymorphonuclear leukocytes. The reason for this was apparent in most of the succeeding blocks, in the presence of many partially encysted larvae of Trichinella. A severe myositis due to these parasites was found in the muscles of the upper part of the arm. The lymph nodes of the elbow were in view in blocks 22 to 24. Many sections were cut from these blocks and no carcinoma was seen. Sections from all of the bones removed from the blocks of the forearm were studied and no carcinoma was found. Therefore, as no carcinoma was found above the wrist, it was concluded that the metastases in the axilla were due to lymphatic embolism, and that this study had demonstrated that metastasis of carcinoma may occur without continuous growth, whether this is the usual method or not.

A Study of Muscle Temperature During Bacterial Chill. A. J. Nedzel (introduced by William F. Petersen), University of Illinois.

By direct measurements of the temperature in the abdominal aorta, rectum and skeletal muscles, the temperature response to the intravenous injection of Bacillus coli has been followed. In the majority of experiments the temperature rose slowly in the abdominal aorta and the rectum while in the muscles there was a definite decrease in temperature. The conclusion is reached that the elevation of temperature in the circulating blood does not depend on the heat produced in the muscles during the period of chill.

BUFFALO PATHOLOGICAL SOCIETY

Regular Meeting, Feb. 23, 1934

Kornel Terplan, *President, in the Chair*

Oxyuriasis of the Appendix. Margaret Warwick.

During the past five years at the Millard Fillmore Hospital 3,196 appendixes were removed. In 1,911 of these the lumens showed contents so preserved that examination for oxyurids could be satisfactorily made. Parasites were found in 35, or 1.8 per cent. By years the incidence has increased from 1 per cent in 1929 to 2 per cent in 1934. The ages of the patients varied from 7 to 34 years, with an average of 17.8 years. That appendical oxyuriasis is not entirely an infestation of childhood was shown by the fact that 51 per cent of the cases were found in patients over 16 years of age, 31 per cent in patients over 20 years and 8.5 per cent in patients over 30 years.

In 60 per cent of the cases in which oxyurids were found the appendix alone was removed; in 40 per cent appendectomy was incidental to operations on other tissues; one appendix was contained in a hernial sac. The symptoms for which appendectomy was done varied from a light attack of abdominal pain lasting over a long period of time to acute attacks of from one to two days' duration. Leukocyte counts were done in fifteen cases. In 53 per cent of these the count showed an increase of from 10,000 to 28,000. For the most part the temperature did not exceed 99 F.; in six cases, or 17 per cent, the temperature reached 100 F.; in two others, in which there were acute appendicitis and pyelitis, the temperature was higher.

In only one case did the appendix show signs of acute inflammation; the parasite was in the lumen surrounded by pus. A few appendixes showed indentations of the mucosa, but no erosions or ulceration. In three, the parasite had penetrated into the mucosal coat. There was no surrounding cellular reaction. Gordon concluded that such a penetration occurred after removal of the appendix. Two of the parasites were surrounded by red blood cells in the lumen. The location of the hemorrhage, however, between the mucosa and a central mass of fecal material suggested a traumatic origin associated with removal. In most cases the worms lay in the lumen, either alone or in a mass of fecal material.

An attempt at correlation of the clinical symptoms and the position of the worm within the lumen showed that the acute attacks of pain were usually associated with the presence of unusually large or numerous parasites in empty lumens. This suggests that, as Aschoff thought, the movements of the oxyurids might cause painful contraction of the appendical wall which could produce symptoms resembling acute appendicitis. It is difficult, however, to believe that contraction could have a relation to increase in the leukocyte count.

The presence of the oxyurids in appendixes is usually an incidental finding. The worms are probably never the cause of appendical inflammation. They may produce painful muscular contractions which invoke symptoms simulating acute appendicitis.

LIPOID HISTIOCYTOSIS WITH TUMOR-LIKE INFILTRATION OF THE SUBCUTANEOUS STRUCTURES OF THE TRUNK. K. TERPLAN, R. S. HUBBARD, C. S. RYERSON, W. J. ROSE AND S. L. VAUGHAN.

A white woman, 35 years of age, was admitted to the Buffalo General Hospital, Sept. 12, 1933, complaining of polydypsia, polyuria and headache of about five years' duration. For two years she had noticed a mass in the right breast, which increased progressively in size, and rapidly growing masses in the neck, left breast and abdomen. There was no exophthalmos. Repeated cholesterol determinations on the blood were within normal limits. Roentgen films of the skeleton were negative. The clinical impression was: Hodgkin's disease or carcinoma of the breasts. The patient died Dec. 9, 1933.

Biopsy of a specimen taken from the mass in the left breast showed no evidence of neoplasm. The picture pointed to a severe metabolic disturbance of the subcutaneous structures with unusual storage of lipoid.

Hematologic examination disclosed a normocytic hyperchromic type of anemia without signs of regenerative activity. Later examinations revealed a progressive anemia with evidences of marked erythropoiesis, especially an erythroblastic crisis; the total leukocyte count fell, with a distinct shift to the left in the neutrophilic nuclear configuration.

Various portions of the swelling removed from the left breast were studied by chemical methods. Attention was centered on the lipoid constituents, and the composition of the stroma was ignored. The lipoids were apparently wholly contained in an acetone extract, for although a second portion soluble in alcohol but insoluble in acetone was obtained, it was also wholly soluble in water but insoluble in ethyl ether and petroleum ether. The relationship between the nitrogen and the phosphorus content of this mixture did not suggest that an alcohol-soluble phospholipid was present.

The material soluble in acetone was also wholly soluble in ethyl ether and practically completely soluble in petroleum ether. It consisted largely of cholesterol ester, together with a small amount of free cholesterol and a trace of phospholipid. There was also present some neutral fat, as well as, apparently, some free fatty acid. The evidence in favor of free fatty acid was: 1. A definite titration value was obtained when a benzene solution prepared from the acetone extract was titrated with alkali. 2. After exact or partial neutralization with alkali followed by extraction and determination of the fatty acid the amount recovered was proportional to the amount of alkali initially added. 3. After cholesterol ester had been removed by crystallization from methyl alcohol a small crop of crystals and amorphous globules was obtained by treatment with ethyl alcohol. These crystals had a very low melting point, did not rotate polarized light, were acid in reaction and stained light yellow with sudan III.

The cholesterol ester, together with a part of the free cholesterol, was obtained in crystalline form by treatment with methyl alcohol. The proportion between the total weight of the crystalline material and that of the cholesterol in the ester form present corresponded with the theoretical figures for cholesterol stearate or cholesterol oleate. By purification with digitonin and repeated purification, first from ether and then from methyl alcohol, a preparation melting sharply between 41 and 42 C. was obtained. Synthetic cholesterol oleate, according to Hurthel, melts at 42 C.

Post mortem there were found: extreme pallor of the entire integument; seborrheic dermatitis limited to the scalp; marked caries of the teeth; nodular lumpy and diffuse lipoid masses of both lateral thoracic walls extending into the axillae, both cervical regions and the submental areas, almost the entire back, and about the upper half of the abdominal wall (deepest depth, 8 cm.); lipoid infiltration of muscles; moderate swelling of cervical lymph nodes with localized caseation; lipoid infiltration in the mediastinum and in the retroperitoneal and pelvic fat tissues; localized lipoid infiltration of the dura mater with thrombosis of several sinuses, and hemorrhagic malacia in the brain; distinct lipoid deposits in the fat marrow of the long bones, and to a slight extent in the short bones (skull not

involved); extensive thrombosis of the iliac and hypogastric veins extending into the superior vena cava; complete thrombosis of the splenic vein, with extensive anemic infarction; thin whitish shreds on the entire mesentery suggestive of organized fibrinous exudate; atrophy of the thyroid gland (6.5 Gm.); normal lipoid-rich suprarenals; very slight atheromatosis.

Histologic examination of subcutaneous tissue pointed to a gradual replacement of the fat by huge foamy cells stored with double-refracting lipoid, and to a reactive inflammation, in places leading to formation of granulation tissue. With sudan III, Nile blue sulphate, Weigert-hematoxylin, Lorrain-Smith and Fischler stains, most of the lipoid consisted apparently of cholesterol oleates. Study of the borders of large areas of foamy cells where small fat islets were still preserved suggested the appearance of double-refracting lipoids in structurally normal fat cells. No lipoid storage could be made out in the endothelium or reticulum cells of the spleen, liver or lymph nodes. Of pathogenic interest was the practically complete fibrous replacement of the posterior lobe of the pituitary gland. The histologic changes in the lungs were similar to those found in some cases of the Christian-Schüller syndrome. The bone marrow, along with the lungs, showed huge foamy cells in sections examined. It is interesting to note that at no time was the blood cholesterol increased.

We were able to find only one case reported in which the subcutaneous tissue was involved by massive lipoid deposits. In 1909 under the title of "Multiple Myxo-Cholesto-Lipomata," Proescher and Meredith described the presence of multiple yellowish tumor masses in the subcutaneous tissues of the leg, breasts and abdomen of a 32 year old woman. From chemical analysis it was concluded that the foreign material was abnormally stored cholesterol fatty acid esters, but the malady was regarded as a tumor process. However, the patient had no diabetes insipidus.

NOTE.—It is intended to publish a more detailed description of this case at a later date.

Book Reviews

Bergey's Manual of Determinative Bacteriology: A Key for the Identification of Organisms of the Class Schizomycetes. By David H. Bergey. Assisted by a committee of the Society of American Bacteriologists. With an index by Robert S. Breed. Fourth edition. Cloth. Price, $6. Pp. 664. Baltimore: Williams & Wilkins Company, 1934.

Following the preface, the chief divisions of the contents of the recently published fourth edition of Bergey's manual are: (1) introduction; (2) an abridged key of the bacterial families, tribes and genera; (3) a section "How Bacteria Are Named and Identified," by Prof. R. E. Buchanan; (4) the latest bacteriologic code; (5) descriptions of species of bacteria, which comprise the bulk of the book, and (6) the index, prepared by Prof. R. S. Breed.

The introduction contains a slightly revised version of the previously published suggestions for the use of the manual in classifying unknown organisms.

The abridged key, adapted from the "Manual of Microbiology" of Obold and Diehm, summarizes the principal characters of the different genera of bacteria. It should be of considerable aid in the allocation of a particular species.

Professor Buchanan's essay "How Bacteria Are Named and Identified," written especially for this edition, compresses into twelve pages a clear and stimulating discussion of the principles of taxonomy and nomenclature. It contains statements of broad biologic concepts, together with a great deal of practical information on the scientific names of the bacteria. The discussion, on page 18, of the concept of species in relation to R and S types, filtrable stages, dissociation phenomena, variability and possible life cycles of the bacteria is particularly interesting and is good evidence of the broadening of the taxonomic point of view. Professor Buchanan notes that there has not been any international agreement as to what stage of a bacterium should be designated the mature, adult or perfect stage and that hence there are uncertainties in classification. In fact, all the stages of bacterial development are not yet known, although the records of variability are becoming increasingly extensive. Only a relatively small part of the existing knowledge of bacterial variation has been incorporated in this edition of the manual. It is satisfactory, however, to read that this deficiency is recognized and that "in future editions it is hoped that this lack may be corrected, and descriptions of variants or stages included."

The latest bacteriologic Code is the outcome of the deliberations of the International Society of Microbiology and the International Botanical Congress in 1930. As it is now recognized that the bacteria have characteristics which differentiate them in several respects from both plants and animals, the International Society of Microbiology voted to follow the rules of nomenclature agreed on by international congresses of botany and zoology only "in so far as they may be applicable and appropriate."

According to the count made by the reviewer, this edition of the manual contains descriptions of 1,179 species of organisms (bacteria, actinomycetes and spirochetes), listed in 114 genera. Two new genera have been recognized, namely, the genus Brucella, for the organisms of infectious abortion, undulant fever and Malta fever, and the genus Listerella, containing the single species monocytogenes, the cause of infectious mononucleosis in rabbits. The placing of the abortus-melitensis organisms in a separate genus, appropriately named, is an improvement on their former unnatural assignment to the genus Alcaligenes, in which they were grouped with the unrelated species faecalis alcaligenes. The genus Pfeifferella, containing the bacillus of glanders has been combined with the genus Actinobacillus, including the bacterium of Lignières and Spitz. This seems to be an unnatural grouping, from the point of view of pathology. According to the preface, about

50 new species have been included, while several organisms have been omitted as distinct species and their names recognized as synonyms for other species.

The instability of nomenclature is exemplified by these shiftings of genera and species and by other editorial as well as taxonomic changes. With the aid of Professor van Eseltine, much has been done to bring "the spelling and endings of scientific names into harmony with the latest international rules" and with Latin grammar. Unfortunately, new names are thus created, and others are to be anticipated. There is still a variation in the use of diphthongs. The genus Hemophilus is thus spelled with phonetic simplicity, but Streptococcus anhaemolyticus retains the ancient orthographic stem.

Professor Breed's index is of immense assistance to both the experts in the Bergey neologisms and those who adhere to the old familiar terminology in finding the locations of descriptions of bacterial species. The index is an almost complete synonymy.

Opinion on the validity of the classifications and groupings used in this manual will vary according to the points of view of bacteriologists. The distinctions of species among the streptococci are doubtful. Bacterium tularense appears to be related more nearly to the organisms of the abortus-melitensis group than to the Pasteurella group, to which it is assigned in this book. Undoubtedly, the membership of many groups might be the subject of prolonged debate, with inconclusive results.

Several omissions are notable. The book does not contain descriptions of the spirillum or spirochete of rat-bite fever, the bacillary forms known as Bartonella and the bacteria-like organisms now called Rickettsia.

In these days of increasing and confusing knowledge of variation, dissociation and antigenic composition, it is probable that most bacteriologists will agree that the bacteria are unclassifiable. On the other hand, they recognize the need of some systematic framework on which to hang, if only temporarily, the shreds of information which are sorted from the sheets of the publications and reports of research. This manual has satisfied that need and has served as an accessible storehouse of older knowledge. The danger exists that the manual, by making a convenient nomenclature available, will tend to fix false notions through everyday usage of names which appear to mean more than they can mean. The uncritical uses of the manual will be exposed to this danger. The editors themselves, however, expressing a liberal point of view and acknowledging that the manual has no "legal" authority, have by their repeated rearrangement of species and genera and their frequent rechristenings of the bacteria, given notice to the reader that each edition has somewhat the characteristics of a progress-report.

The writings of bacteriologists both in this country and abroad indicate that the manual is being used with increasing frequency. This edition was awaited with keen anticipation by many students, and its appearance, with the revised descriptions, is welcomed as a most valuable source of information and an instrument of great service to the science of bacteriology.

The use of a better grade of slightly glazed paper has made the type more legible, improved the appearance of the book and made it more substantial than any of the previous editions.

Recent Advances in Pathology. By Geoffrey Hadfield, M.D., F.R.C.P. (Lond.), Professor of Pathology in the University of Bristol, Examiner in Pathology, Late Professor of Pathology, in the University of London, and Lawrence P. Garrod, M.A., M.D., B.Ch. (Camb.), M.R.C.P. (Lond.), Bacteriologist and Lecturer in Bacteriology, Late Demonstrator of Pathology, St. Bartholomew's Hospital. Second edition. Price, $4. Pp. 457, with 69 illustrations. Philadelphia: P. Blakiston's Son & Co., 1934.

The first edition of this book, which appeared two years ago, received favorable mention in the ARCHIVES (**14**:589 [Oct.] 1932). The new edition has been revised and enlarged. Among the minor additions may be mentioned statements and discussions concerning thorotrast, monocytic leukemia, cancer caused by radium,

filtrable tumors, resistance to cancer, silicosis, the frequency of cancer of the lung and the results of newer observations on the suprarenal gland in Addison's disease. There is a new section on the pituitary gland, in which the recent advances in its study are presented. The chapter on the liver has been extended by a new section on the recent work on the formation of gallstones. Peptic ulcer and the relations of gastro-intestinal disorders to anemia receive revised and new consideration. The structural changes in the deficiency diseases are well described in the new edition. The chapters on Bright's disease have been rearranged and expanded. It is not practicable to review the seventeen chapters one by one in detail. The book may be recommended as giving convenient, reliable, helpful summaries of recent investigations of the reticulo-endothelial system; tissue culture; cancer; the structural changes in deficiency diseases and important problems connected with the cardiovascular, respiratory, digestive, urinary and nervous systems and the ductless glands.

Industrial Toxicology. By Alice Hamilton, M.D. Harper's Medical Monographs. Fabrikoid. Price, $3. Pp. 352. New York: Harper & Brothers, 1934.

This book aims "to present what is most needed in a short review of modern industrial toxicology rather than a logically planned textbook." The introduction deals with the general methods of preventing industrial poisoning, particularly as concerns the duties and responsibilities of the physician. That the worker in chemical industry shall benefit as much as possible from what is known about industrial poisoning, its prevention and treatment is the basic purpose of the author. There are ten chapters treating of the following: alkalis; acids; chromium; lead; arsenic; mercury; metal-fume fever; other metals (copper, phosphorus, zinc, etc.); asphyxiants (carbon monoxide and dioxide, hydrogen sulphide and cyanide); benzene and other derivatives of coal tar; petroleum and derivatives; turpentine; carbon disulphide; tobacco; mineral oils; occupational cancer, and radio-active substances. There is an extensive bibliography (to January 1933), which will be of great help. The book is well written. Within a small compass it contains a great deal of reliable information about industrial poisoning. It reveals that industrial chemical methods change, that new chemicals come into use, that old chemicals are used in new ways and that dangers of industrial poisoning continue. The book will be of value to all who are concerned with such problems; it supplements nicely the larger book by the same author ("Industrial Poisons in the United States," New York: The Macmillan Company, 1925).

Books Received

La séroréaction blennorragique. Bertrand Reme. Price, 30 francs. Pp. 179. Paris: Masson & Cie, 1934.

Here is a full account of the history, the technic, the evolution, the specificness and the practical value in diagnosis, prognosis and prophylaxis of complement fixation in gonococcic infection. The bibliography is as complete as possible. Technical matters are discussed in detail, and the importance of standardized, stable antigen is emphasized.

Dysentery in Denmark: A Contribution to the Bacteriology and Epidemiology of Infection with Sonne and Flexner Bacilli. Communications de l'Institut Sérothérapique de l'Etat Danois, tome 24, 1934. Knud Bojlén. Pp. 231. Copenhagen: Bianco Lunos, 1934.

Parasitism and Disease. Theobald Smith, Director Emeritus of the Department of Animal Pathology, Rockefeller Institute for Medical Research. Published by the Louis Clark Vanuxem Foundation. Price, $2. Pp. 196. Princeton: Princeton University Press, 1934.

Industrial Toxicology. Harper's Medical Monographs. Alice Hamilton, M.D. Price, $3. Pp. 352. New York: Harper & Brothers, 1934.

Diseases Peculiar to Civilized Man: Clinical Management and Surgical Treatment. George Crile, M.D. Edited by Amy Rowland. Price, $5. Pp. 427, with 41 illustrations. New York: The Macmillan Company, 1934.